Relationship of Serum Phenylalanine Levels and Ability of Phenylketonurics to Hydroxylate Tryptophan. (24686)

ROBERT C. BALDRIDGE, LEATRICE BOROFSKY, HENRY BAIRD III, FREDERICK REICHLE AND DONNA BULLOCK (Introduced by M. J. Oppenheimer)

Depts. of Physiological Chemistry and Pediatrics, and St. Christopher's Hospital, Temple University School of Medicine, Philadelphia, Pa.

Although there is evidence(1) in phenylketonurics for hereditary lack of a component of the enzyme system which catalyzes conversion of phenylalanine to tyrosine, the cause of associated mental retardation remains unknown. Increased excretion of non-phenylalanine metabolites such as indoleacetic acid and indolelactic acid by uncontrolled phenylketonurics has been reported(2), and Pare *et al.*(3) noted lower levels of 5-hydroxytryptamine (serotonin) in serum and decreased amounts of 5-hydroxyindoleacetic acid (5-HIAA) in urine of uncontrolled phenylketonurics compared with similar data for normal and non-phenylketonuric mentally retarded children. The latter workers suggested decreased formation of serotonin may be related to mental defect. The concept has been developed that increased concentrations of phenylalanine (or related metabolites) in blood and tissues of phenylketonurics may competitively inhibit the hydroxylase system which catalyzes conversion of tryptophan to 5-hydroxytryptophan, the precursor of serotonin. Decreased rate of formation of serotonin is reflected in decreased excretion of its major metabolic end product, 5-hydroxyindoleacetic acid. If such a concept is correct, patients with high blood phenylalanine levels would be expected to excrete more 5-hydroxyindoleacetic acid following transition to a low-phenylalanine diet (with concomitant decrease in phenylalanine levels). Additionally, phenylketonuric patients whose blood and tissue levels of phenylalanine are in normal range, following dietary control, should excrete greater amounts of 5-hydroxyindoleacetic acid than do uncontrolled phenylketonurics. Finally, increasing the ratio of tryptophan to phenylalanine in the diet of the latter might result in increased formation of 5-hydroxylated products. Experiments designed to test these hypotheses form the basis of this report.

Methods. Serum phenylalanine levels were determined by paper chromatographic method of Berry(4). Urinary 5-hydroxyindoleacetic acid was measured essentially as described by Udenfriend *et al.*(5). With each urine sample, parallel analyses were made of aliquots to which were added known amounts of 5-hydroxyindoleacetic acid. Recovery of the latter ranged between 80-90%. Creatinine determinations were performed by the Jaffe alkaline picrate procedure and in some cases by adsorption method (with Lloyd's reagent). Urine collection bottles contained acetic acid as a preservative and contents were frozen and stored at −20°C until analyzed 2-3 days later. Attempts were made to collect 24 hour urine specimens from each patient; however, due to uncertainties of collection, data are presented in terms of ratio of μg of 5-hydroxyindoleacetic acid excreted/mg of creatinine. The low-phenylalanine diet consisted of Lofenalac* plus foods calculated to give daily intake of 15-25 mg of phenylalanine/kilo body weight.

Results. The inverse relationship between serum phenylalanine levels and excretion of 5-hydroxyindoleacetic acid during transition to a low phenylalanine diet is illustrated in Fig. 1 (patient W). An increase in 5-hydroxyindoleacetic acid excretion occurred within 4 days following ingestion of the low-phenylalanine diet.

After he had eaten the low-phenylalanine diet for 4 months (Table I), the 5-hydroxyindoleacetic acid to creatinine ratio of patient W was increased to over 10; a similar increase was noted with a second patient (C). Other patients (D and S) who had been fed the diet for 4 to 10 months excreted amounts of 5-hydroxyindoleacetic acid which, when expressed in terms of ratio of 5-hydroxyindole-

* Lofenalac, complete dietary preparation, was kindly furnished by Mead Johnson and Co.

TABLE I. Relation of Diet, Serum Phenylalanine Levels and Urinary 5-Hydroxyindoleacetic Acid (5-HIAA) of Phenylketonurics.

Patient		Age (mo) at start of diet	Prior to ingestion of low phenylalanine diet: Serum phenylalanine (mg %)	Prior to ingestion of low phenylalanine diet: Urinary 5-HIAA (μg/mg of creatinine)	After ingestion of low phenylalanine diet*: Period fed diet (mo)	After ingestion of low phenylalanine diet*: Urinary 5-HIAA (μg/mg of creatinine) Range	Urinary 5-HIAA (μg/mg of creatinine) Mean
W	♂	30	25	2.0	4	9.9–10.4	10.2†
C	♀	18	50	1.9	10	9.2–11.5	10.4†
D	♀	15	60		2		5.4
					10	9.2–11.1	10.2†
S	♀	25	40		2– 4	10.4–14.8	12.8‡
					7–10	11.2–13.8	11.9‡

* Serum phenylalanine levels were less than 5 mg %. † Mean of 2 determinations, 3-5 days apart. ‡ Mean of 4 determinations, 2-3 wk apart.

acetic acid to creatinine, were also in the same range.

A phenylketonuric (B, age 38 months, a first cousin of patient W) who refused to eat the low-phenylalanine diet, was fed 100 mg of L-tryptophan/kg body weight on 5th and again on 8th day after admission to hospital. There was an immediate increase in excretion of 5-hydroxyindoleacetic acid following administration of tryptophan (Fig. 2). Excretion decreased on 10th day to the lower level (ratio of 2-3) typical of uncontrolled phenylketonurics.‡ Patient B later was fed 100 mg of 5-hydroxytryptophan† daily along with normal diet. Between 10 and 15% of the amount fed was excreted in urine as 5-hydroxyindoleacetic acid (measured by Udenfriend procedure). Assessment of the value of 5-hydroxytryptophan administration must be postponed. However, preliminary testing revealed a spurt in mental development in (B) who was given the latter compound. His serum phenylalanine levels ranged from 20-25 mg % during the period studied.

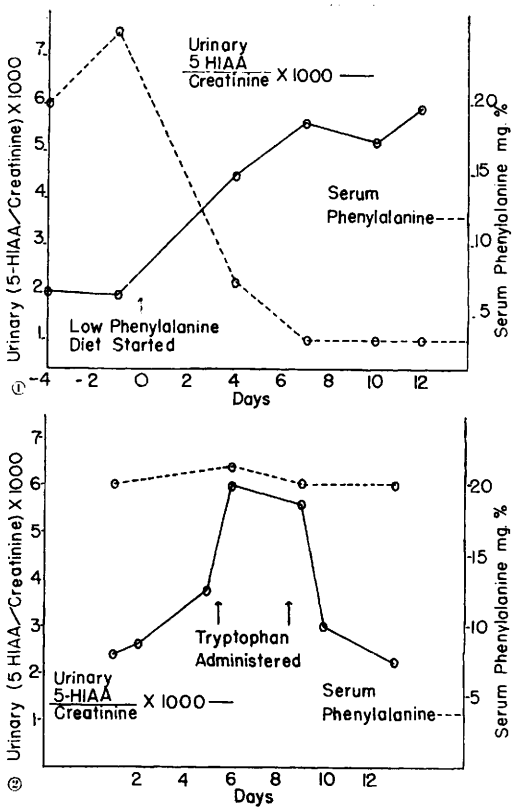

FIG. 1. Changes in serum phenylalanine levels and urinary 5-hydroxyindoleacetic acid (5-HIAA) excretion during transition to a low-phenylalanine diet.

FIG. 2. Effect of administration of tryptophan on 5-hydroxyindoleacetic acid excretion by uncontrolled phenylketonuric.

Discussion. Patients studied by Pare *et al.* (3) were older than those described here. However, urinary 5-hydroxyindoleacetic acid/creatinine ratios reported for their group were similar to those noted in our patients prior to dietary control. After present studies had been completed, Pare's group reported(6)

‡ The feeding of equivalent amounts of L-tryptophan to two phenylketonurics who had been eating a low phenylalanine diet (serum phenylalanine levels less than 5 mg %) was *not* followed by such an increase in 5HIAA excretion.

† We are indebted to Dr. Gustav J. Martin, National Drug Co., for supplies of 5-hydroxytryptophan,

that phenylketonurics serum serotonin levels were significantly higher after ingestion of a low-phenylalanine diet for 4 weeks than before dietary control. Their failure to note significant differences in excretion of 5-hydroxyindoleacetic acid in the time studied, was attributed to use of random rather than 24-hour samples of urine. Ages of patients studied were not given. While our studies were in progress, Berendes *et al.*(7) reported that phenylketonurics who had been fed low-phenylalanine diets excreted more 5-hydroxyindoleacetic acid/24 hours than did untreated phenylketonurics. Creatinine values were not given.

Summary. 1. Following a decrease in serum phenylalanine levels (induced by feeding of a low-phenylalanine diet) phenylketonurics excrete increased amounts of 5-hydroxyindoleacetic acid. 2. The ratio of urinary 5-hydroxyindoleacetic acid to creatinine is about 5-fold greater for young phenylketonuric children, fed a low-phenylalanine diet for 4-10 months, than for untreated phenylketonurics. 3. Oral administration of L-tryptophan to untreated phenylketonuric was followed by increase in 5-hydroxyindoleacetic acid excretion. 4. Following oral administration of 5-hydroxytryptophan, 10-15% was excreted as urinary 5-hydroxyindoleacetic acid by an otherwise untreated phenylketonuric.

1. Wallace, H. W., Moldave, K., Meister, A., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 632; Jervis, G. A,. *ibid.*, 1953, v82, 514.

2. Armstrong, M. D., and Robinson, K. S., *Arch. Biochem. and Biophys.*, 1954, v52, 287.

3. Pare, C. M. B., Sandler, M., Stacey, R. S., *Lancet*, 1957, v272 i, 551.

4. Berry, H. K., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 71.

5. Udenfriend, S., Titus, E., Weissbach, H., *J. Biol. Chem.*, 1955, v216, 499.

6. Pare, C. M. B., Sandler, M., Stacey, R. S., *Lancet*, 1958, v273 ii, 1099.

7. Berendes, H., Anderson, J. A., Priggie, B., Ruttenberg, D., Ziegler, M. R., *Staff Meeting Rept., Univ. of Minn. Med. Bull.*, 1958, v29, 498.

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Production of Leucocytic Exudates in Rat Granuloma Pouch.* (24687)

GUILLERMO SOBERON† AND PHILIP P. COHEN
Dept. of Physiological Chemistry, University of Wisconsin, Madison

A relatively simple procedure for producing inflammatory exudates rich in leucocytes, based on rat granuloma pouch technic of Selye *et al.*(1,2,3) is reported in this paper. Several technics for production of sterile inflammatory exudates have been reported. Opie(4,5,6,7) used aleuronat and starch for production of sterile exudates in the pleural and peritoneal cavities of dogs; Ponder and MacLeod(8) employed intraperitoneal saline injection method in rabbits. Menkin(9) employed turpentine injections for production of sterile pleural exudates in dogs. Technics for separation of leucocytes from whole blood have been reported by Tullis(10). The granuloma pouch technic as developed by Selye and coworkers for study of anti-inflammatory agents(3) appeared to us to offer several advantages for the purposes of our studies. Conditions necessary for producing an inflammatory exudate rich in myeloperoxidase-containing polymorphonuclear leucocytes are here reported.

Methods. Male, Sprague-Dawley rats were used. *Formation of pouch.* After ether anesthesia, the back of the animal was shaved with fine electric clippers and 1% iodine solution applied on the skin. Forty to 60 ml of air were introduced subcutaneously by means of a syringe and 22 gauge needle. The pouch usually covered about ¾ of the back of the

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† Fellow of E. R. Squibb and Sons, Mexico, S. A. Present address, Dept. of Biochemistry, Hosp. de Enfermedades de la Nutricion, Mexico City. D.F.