pounds than those described here. Induction of fibrinolytic activity in human plasma by addition of such compounds may lead to new approaches for development of thrombolytic agents.

Summary. A number of urea derivatives added at low concentrations to plasma before or after clotting induce spontaneous dissolution of human plasma clots. They also considerably enhance the activity of urokinase. Ethyl urethan was the most active compound of the group. Bovine fibrin clots are dissolved by ethyl urethan only in presence of human plasma.

1. von Kaulla, K. N., *Nature,* 1959, v184, 1320.
2. Palitzsch, S., *Biochem. Z.,* 1915, v70, 333.
3. Lorand, L., *Nature,* 1950, v166, 694.
4. Juehling, L., Woehlisch, E., *Biochem. Z.,* 1938, v298, 312.
5. Ratnoff, O. D., *J. Exp. Med.,* 1948, v87, 199.
6. von Kaulla, K. N., *Nature,* 1949, v164, 408.

Received December 9, 1960. P.S.E.B.M., 1961. v106.

Metabolism of 5-Hydroxyindole Compounds in Experimentally Produced Phenylketonuric Rats.*† (26393)

IRENE HUANG, SHEILA TANNENBAUM, LUCY BLUME AND DAVID YI-YUNG HSIA
Genetic Clinic, Children's Memorial Hospital, and Dept. of Pediatrics, Northwestern University Medical School, Chicago, Ill.

The studies of Jervis(1) have shown that the primary biochemical defect in phenylketonuria consists of an inability to oxidize phenylalanine to tyrosine due to absence of the liver enzyme, phenylalanine hydroxylase. This results in an accumulation of phenylalanine in the blood and excretion of phenylketones in the urine. Armstrong and Robinson (2) and Ferari *et al.*(3) have demonstrated the presence of indolelactic acid in urine of phenylketonuric patients and suggested that there may also be a disorder of tryptophane metabolism in this disease. More recently, 3 different groups(4-6) have observed a significant lowering of 5-hydroxytryptamine in the serum and of 5-hydroxyindoleacetic acid in the urine of phenylketonuric patients. These changes are reversible when the patients are placed on a diet low in phenylalanine content. Pare *et al.*(7) have suggested that this disturbance of 5-hydroxyindole metabolism in phenylketonuria is due to an inhibition of 5-hydroxytryptophan decarboxylase by phenylalanine metabolites, a finding which has been confirmed by both *in vitro*(8) and *in vivo*(9,10) studies.

Auerbach *et al.*(11) showed that phenylketonuria could be produced experimentally in rats by feeding excessive amounts of both phenylalanine and tyrosine. This provided a means for studying the metabolism of the 5-hydroxyindole compounds in an *in vivo* system which is genetically normal.

Materials and methods. Phenylketonuria was produced experimentally according to the procedure described by Auerbach *et al.* (11). Weanling rats of the Sprague-Dawley strain were placed on stock diets and experimental diets as follows: 1) 3.75% L-tyrosine and 3.75% DL-phenylalanine for 3 months, 2) 3.75% L-tyrosine and 3.75% DL-phenylalanine for 2 months, 3) 3.75% L-tyrosine and 2.5% DL-phenylalanine for 2 months, and 4) 2.5% DL-phenylalanine alone for 2 months.

Twenty-four hour collections of urine were obtained weekly and phenylpyruvic acid was determined by the method of Berry and Woolf(12) and 5-hydroxyindoleacetic acid by the method of Udenfriend *et al.*(13). Blood samples were obtained by cardiac puncture at 6½ to 7 weeks and 9 weeks after start of the diet. The plasma was analyzed for 5-hydroxytryptamine using the method

* Aided by grants from Illinois Mental Health Fund and Public Health Service.

† The authors wish to express their appreciation to Dr. Harry Waisman for many helpful suggestions.

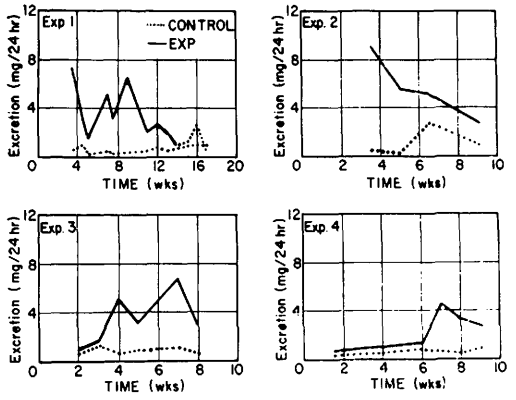

FIG. 1. Mean urinary phenylpyruvate excretion expressed as mg/24 hr for rats fed stock and experimental diets.

Exp. 1. 3.75% L-tyrosine + 3.75% DL-phenylalanine.
Exp. 2. 3.75% L-tyrosine + 3.75% DL-phenylalanine.
Exp. 3. 3.75% L-tyrosine + 2.5% DL-phenylalanine.
Exp. 4. 2.5% DL-phenylalanine.

described by Waalkes(15) and phenylalanine by the spectroscopic method of LaDu and Michael(14). Because of the high levels of tyrosine in the experimental rats, the plasma from these animals was first chromatographed with n-butanol saturated with 2N NaOH, the section of the filter paper containing tyrosine removed, and the remaining fraction eluted with distilled water and concentrated before being used for determination of phenylalanine.

Liver phenylalanine hydroxylase was determined by the method of Kenney *et al.* (16). One milliliter of soluble fraction of liver prepared from 33% homogenates in 0.15 M KCl by centrifugation in a preparatory ultracentrifuge at 10,500 g for 30 minutes was shaken at 35°C for 60 minutes with 150 μM of potassium phosphate buffer (pH 6.8) 2 μM of phenylalanine, 2 μM of DPNH, and 5 μM of nicotinamide in a total volume of 1.75 ml. At the end of incubation, 0.25 ml of water was added, and protein was precipitated by 0.5 ml of 30% trichloroacetic acid. 0.2 ml of clear supernatant was used for tyrosine determination by the colorimetric method of Udenfriend and Cooper (11). Protein content of each homogenate was determined by measuring the absorption at 260 and 280 mμ, and calculated as follows: Protein concentration (mg/ml) = 1.55 O.D.$_{280}$ − 0.76 O.D.$_{260}$. Amount of enzyme activity was expressed as μM of tyrosine formed per 100 mg of protein.

Results. Mean values of the 24 hour excretion of phenylpyruvate are given in Fig. 1. The majority of the experimental animals placed on both phenylalanine and tyrosine were found to excrete increased amounts of phenylpyruvate as soon as sufficient amounts of urine could be obtained for analysis. This increased to a peak level of 5 to 6 mg/24 hours at 7 to 9 weeks after being placed on the diets. Phenylpyruvate excretion reverted to control levels when phenylalanine and tysosine supplementation was discontinued. When the rats were fed 2.5% DL-phenylalanine alone, there was a delay in increase of phenylpyruvate excretion until the animals had been on the diet for 4 to 6 weeks.

Plasma phenylalanine levels obtained at 6½ weeks to 7 weeks and at 9 weeks showed a similar and statistically significant rise in experimental animals fed phenylalanine and tyrosine (Table I). However, the animals fed 2.5% DL-phenylalanine alone failed to show a significant increase.

Mean values of urinary 5-hydroxyindoleacetic acid excretion in these same animals are shown in Fig. 2. There was a decrease in experimental animals fed tyrosine and phe-

TABLE I. Blood Phenylalanine Levels Expressed as μg/ml in Rats Fed Stock and Experimental Diets.

Diets	Weeks on diet 6½ to 7	9
Control	18.6 ± 9.8 (3)	8.8 ± 6.0 (4)
3.75% L-tyrosine & 3.75% DL-phenylalanine	34.6 ± 6.2 (3)	33.1 ±13.3 (4)
Control	12.7 ± 7.1 (2)	7.3 ± 6.7 (5)
3.75% L-tyrosine & 2.5% DL-phenylalanine	42.4 ±14.0 (5)	32 ±13.9 (4)
Control	9.3 ± 4.8 (4)	...
2.5% DL-phenylalanine	10.5 ± 1.9 (3)	—

Numbers in parentheses indicate No. of animals sacrificed in each experiment. Values expressed as means and stand. dev.

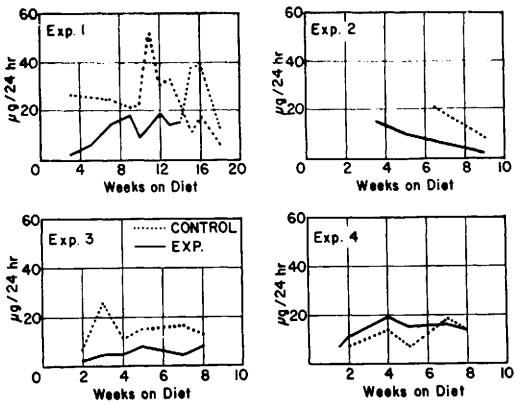

FIG. 2. Mean urinary 5-hydroxy indoleacetic acid excretions expressed as μg/24 hr for rats fed stock and experimental diets.

Exp. 1. 3.75% L-tyrosine + 3.75% DL-phenylalanine.

Exp. 2. 3.75% L-tyrosine + 3.75% DL-phenylalanine.

Exp. 3. 3.75% L-tyrosine + 2.5% DL-phenylalanine.

Exp. 4. 2.5% DL-phenylalanine.

nylalanine and a close correlation existed between rise of phenylpyruvate and fall of 5-hydroxyindoleacetic acid. No such decrease could be found in the animals given 2.5% DL-phenylalanine alone.

Plasma 5-hydroxytryptamine levels at 6½ to 7 and 9 weeks are shown in Table II. There was a significant decrease noted in the experimental animals fed phenylalanine and tyrosine. However, no such difference could be observed among the animals given 2.5% DL-phenylalanine alone.

TABLE II. Blood 5-Hydroxytryptamine Levels Expressed as μg/ml in Rats Fed Stock and Experimental Diets.

Diets	Weeks on diet 6½ to 7	9
Control	.88 ± .08 (5)	.81 ± .22 (5)
3.75% L-tyrosine & 3.75% DL-phenylalanine	.48 ± .10 (5)	.42 ± .09 (5)
Control	.86 ± .18 (10)	.83 ± .17 (10)
3.75% L-tyrosine & 2.5% DL-phenylalanine	.58 ± .12 (10)	.51 ± .15 (10)
Control	.55 ± .13 (5)	.70 ± .21 (5)
2.5% DL-phenylalanine	.57 ± .08 (5)	.66 ± .31 (5)

Numbers in parentheses indicate No. of animals sacrificed in each experiment. Values expressed as means and stand. dev.

Discussion. From these data it would appear that phenylketonuria can be experimentally produced in the rat by supplementation of both phenylalanine and tyrosine to the diet. This effect is not nearly as marked when phenylalanine alone is administered. Auerbach *et al.*(11) have suggested that simultaneous administration of tyrosine might cause an adaptive decrease of phenylalanine hydroxylase in the liver. We have repeated these studies and found enzyme activity to be decreased only slightly (Table III). This discrepancy may in part be caused by differences in the methods used for assay of phenylalanine hydroxylase in the two studies.

The present data demonstrate that the decrease of 5-hydroxyindole compounds in phenylketonuria is a secondary result of excessive phenylalanine levels, and does not represent a second genetically determined metabolic lesion. This is shown by taking a normal animal, producing a metabolic lesion resembling phenylketonuria, and getting a decrease of the 5-hydroxyindole compounds. Finally, removal of the added phenylalanine and tyrosine from the diet resulted in a reversal to normal. The possible role of phenylalanine compounds upon 5-hydroxytryptophan decarboxylase in the intact animal is currently being investigated.

The etiology of the mental defect in phenylketonuria is unknown. However, it has been shown that if such children are treated with a low phenylalanine diet at an early age, near-normal mental development will result. A similar course of treatment in an older affected child is of little or no value(18). Recently, we(19) have reported on developmental pattern of 5-hydroxytryptophan decarboxylase in the newborn rat kidney. There is an absence of the enzyme in the fetal and newborn rat, and activity increases gradually to adult levels by 33 days of age. One could speculate that the excessive amounts of phenylalanine in the phenylketonuric child would further inhibit an already immature enzyme system, and this in turn

TABLE III. Liver Phenylalanine Hydroxylase Activity Expressed as μM Tyrosine Formed/100 mg Protein in Rats Fed Stock and Experimental Diets.

Wk on diet	Controls	3.75% L-tyrosine & 2.75% DL-phenylalanine	3.75% L-tyrosine & 2.5% DL-phenylalanine	2.5% DL-phenylalanine
1	4.42 ± .94 (7)	3.25 ± .82 (7)	—	—
2	4.44 ± 1.10 (7)	3.46 ± .84 (7)	—	—
3	4.39 ± 1.30 (7)	4.34 ± 1.10 (7)	—	—
4	4.73 ± 1.10 (7)	3.27 ± .41 (5)	—	—
6½ to 7	4.33 ± 1.60 (8)	4.11 ± .90 (5)	4.08 ± .27 (5)	5.31 ± 2.36 (5)
9	4.94 ± 2.15 (10)	3.48 ± .89 (6)	4.18 ± .86 (5)	5.05 ± 2.31 (5)

Numbers in parentheses indicate No. of animals sacrificed in each experiment. Values expressed as means and stand. dev.

will result in a decrease of 5-hydroxytryptamine which is essential to the developing brain.

Summary. Data have been presented confirming experimental production of phenylketonuria in rats. Supplementation of the diet with both phenylalanine and tyrosine resulted in a marked increase of phenylpyruvate excretion in urine and of phenylalanine level in plasma. Supplementation with 2.5% DL-phenylalanine alone showed a much less marked effect. The rise of phenylpyruvate coincided with a decerase of 5-hydroxyindoleacetic acid in the urine. Similarly, the increase of phenylalanine in the plasma coincided with a decrease of 5-hydroxytryptamine in the plasma. This indicates that the disturbance of 5-hydroxyindole metabolism in phenylketonuria is secondary to the increase in phenylalanine. The possible significance of these phenomena in the etiology of mental deficiency and phenylketonuria has been discussed.

1. Jervis, G. A., PROC. SOC. EXP. BIOL. AND MED., 1953, v82, 514.

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

3. Ferari, V., Campagnari, F., Guida, A., *Minerva Med.*, (Pavia), 1955, v46, 119.

4. Pare, C. M. B., Sandler, M., Stacey, R. S., *Lancet*, 1957, v1, 551.

5. Berendes, H., Anderson, J. A., Ziegler, M. R., Ruttenberg, D., *J. Dis. Child.*, 1958, v96, 430.

6. Baldridge, R. C., Borofsky, L., Baird, H., Reichle, F., Bullock, D., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 529.

7. Pare, C. M. B., Sandler, M., Stacey, R. S., *Arch. Dis Child.*, 1959, v34, 422.

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

9. Sandler, M., Close, H. O., *Lancet*, 1959, v2, 316.

10. Davies, A., Rimington, C., *ibid.*, 1959, v2, 318.

11. Auerbach, V. H., Waisman, H. A., Wyckoff, L. B., *Nature*, 1958, v182, 871.

12. Berry, J. P., Woolf, L. I., *ibid.*, 1952, v169, 202.

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

14. LaDu, B. N., Michael, P. J., *J. Lab. & Clin. Med.*, 1960, v55, 491.

15. Waalkes, T. P., *ibid*, 1959, v53, 824.

16. Kenney, F. T., Reem, G. H., Kretchmar, N., *Science*, 1958, v127, 86.

17. Udenfriend, S., Cooper, J. R., *J. Biol. Chem.*, 1952, v196, 227.

18. Knox, W. E., *Pediatrics*, 1960, v26, 1.

19. Huang, I., Tannenbaum, S., Hsia, D. Y. Y., *Nature*, 1960, v186, 717.

Received December 15, 1960. P.S.E.B.M., 1961, v106.