they are completely separable by specific adsorption techniques and by gel filtration. These findings should prove helpful when antibody titers to both of these serological tests are positive in patients with the clinical syndrome of infectious mononucleosis who are being studied for the presence of acute toxoplasmosis(3,4,8).

In the present study, the prevalence of dye test antibody titers did not vary significantly among students whose sera were positive in the heterophile determination when compared with students whose sera were negative in that test. In contrast, in a study at the University of California at Berkeley, there was a less frequent occurrence of positive Toxoplasma dye tests among patients in whom the diagnosis of infectious mononucleosis was confirmed by the findings of a positive heterophile antibody test (6%) as compared with those in whom the latter test was negative (20%)(3). The apparent discrepancy of results in these two studies may be accounted for by the smaller number of students in the California study.

The results obtained in the analytical ultracentrifuge with the fractionated serum proteins agree with those reported by Flodin and Killander(7), who found that "19S" antibodies were eluted in the first peak and the "7S" antibodies in the second one. The component from the first peak with a sedimentation coefficient of about 9S may be comparable to a similar peak described by these same authors who concluded that these materials are lipoproteins. Immunoelectrophoretic analysis of Fraction I did not reveal a precipitin line corresponding to that found with normal serum 7S γ-globulins.

Summary. The prevalence and level of dye test antibodies was found to be comparable in sera from students with and without a positive heterophile test. Dye test antibodies were completely separable from heterophile antibodies present in the same sera by means of specific adsorption techniques and by exclusion chromatography on Sephadex G 200.

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Defective 5-Hydroxylation of Tryptophan in Phenylketonuria.* (28847)

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Knowledge of the exact mechanism by which mental defect is produced in phenylketonuria is not available. A detailed understanding might not only lead to improvements in treatment of this disease, and of other forms of mental deficiency involving disorders in amino acid metabolism, but might also shed light on some fundamental biochemical factors which play a role in mental function.

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Pare *et al*(1) first noted a defect in the 5-hydroxyindole pathway of tryptophan metabolism in phenylketonurics, and suggested that a failure in serotonin production might play some part in the pathogenesis of the mental deficiency. It has since been confirmed that serum serotonin concentrations and urinary excretion of 5-hydroxyindoleacetic acid (5-HIAA) are lower in untreated phenylketonurics than in normal individuals, and that these chemical variations revert towards normal when phenylketonurics are placed on a low-phenylalanine diet(2-5). The serotonin deficiency in phenylketonuria was at first attributed to an inhibition of tryptophan hydroxylase(1,4), and later to an inhibition of aromatic L-amino acid decarboxylase(2,3,6-8) by high tissue concentrations of phenylalanine. We believe that the experiments described below support the thesis that defective 5-hydroxylation of tryptophan does occur in phenylketonurics, and that it may be primarily responsible for the decreased serotonin production in these patients.

Subjects and methods. In a preliminary experiment, urinary excretion of serotonin, tryptamine, and 5-HIAA was measured in 2 mentally defective adult phenylketonuric men, and in 2 control adult male patients with mongolism living on the same hospital ward. All urine collections were made while the subjects were maintained on a strictly plant-free diet, since many fruits are known to contain appreciable amounts of serotonin and might possibly also contain its precursor amino acid, 5-hydroxytryptophan (5-HTP). Serotonin and tryptamine in urine were quantitated by 2-dimensional paper chromatography, after the amines had been concentrated and separated from the other constituents of urine(8). The concentration of 5-HIAA in urine was determined by 2-dimensional paper chromatography after extraction of indoles from acidified urine with ethyl acetate, using a modification of the method of Armstrong *et al*(9). Fasting serum phenylalanine concentrations were determined on the phenylketonurics by a modification of the method of Kapeller-Adler(10).

Urine specimens were collected for 48 hours on these 4 subjects while they consumed a plant-free but otherwise normal diet. At this time both phenylketonurics exhibited markedly elevated serum phenylalanine concentrations and excreted large amounts of phenylpyruvic acid in the urine. Each of the 4 subjects was then given a single large oral load of L-tryptophan (100 mg/kg), and urine was collected for 24 hours. Four days later, a single oral load of 100 mg of DL-5-HTP was given each subject, and urine was again collected for 24 hours. The phenylketonuric subjects were then placed on a low-phenylalanine diet for 4 weeks. When their serum phenylalanine concentrations had fallen to the normal range (3 to 5 mg per 100 ml for the method used), urine was again collected for 48 hours.

In a second experiment, urinary excretion of serotonin, tryptamine, and 5-HIAA was similarly measured in 2 adolescent male phenylketonurics, in 2 adolescent male patients with mongolism, and in a normal adult male. During the second experiment, oral loads of L-tryptophan (100 mg/kg), and of DL-5-HTP (100 mg per subject) were also administered, but urine was collected for only 12 hours after the amino acid load had been ingested, in order to accentuate differences in urinary excretion of metabolites. Since it was not possible to place the 2 phenylketonuric subjects on a low-phenylalanine diet as had been done in the initial experiment, 48-hour urine collections were obtained from 2 additional phenylketonuric infants who were receiving the low-phenylalanine diet therapeutically. The mental defectives during this study all lived on the same hospital ward, while the normal subject was a medical student living at home. All urine specimens were obtained, as before, while subjects consumed a plant-free diet.

Results. Tables I and II present the amounts of serotonin, tryptamine, and 5-HIAA found in urines collected during the 2 experiments. The concentration of these compounds is expressed in relation to the creatinine content of urine, since accurately timed and complete urine collections are difficult to

obtain in ambulatory mental defectives. The actual amounts of serotonin and tryptamine excreted were undoubtedly somewhat greater than indicated in Tables I and II, since experiments in which authentic amines were added to urine indicated that approximately 50% of serotonin and 70% of tryptamine were recovered by the technique used.

Serotonin was present in very low concentration or could not be detected at all in the

TABLE I. Urinary Excretion of Serotonin and Tryptamine (as Free Base), and of 5-HIAA, Under Conditions Described in Text.

Subject	Age (yr)	Condition	Serum phenylalanine (mg/100 ml)	Serotonin (μg/100 mg creatinine)	Tryptamine (μg/100 mg creatinine)	5-HIAA (μg/100 mg creatinine)
			Phenylketonurics			
1	48	Control	33.6	0	5.4	140
		Tryptophan load*		0	15.7	135
		5-HTP load*		12.2	2.9	1,950
		Low-phenylalanine diet	2.8	.9	1.6	325
2	23	Control	36.4	0	3.3	70
		Tryptophan load*		0	11.4	115
		5-HTP load†		1.5	1.4	650
		Low-phenylalanine diet	6.3	.2	2.0	185
			Mongoloids			
3	20	Control		1.2	2.7	155
		Tryptophan load*		4.7	17.5	375
		5-HTP load*		17.5	3.7	1,680
4	20	Control		1.2	3.3	220
		Tryptophan load*		2.5	8.2	530
		5-HTP load*		5.2	7.7	360

* Urine collected for 24 hr after amino acid loading.

† Total creatinine in this specimen indicated that urine formed during approximately 42 hr had been collected, thus diluting the serotonin and 5-HIAA produced by the 5-HTP load.

TABLE II. Urinary Excretion of Serotonin and Tryptamine (as Free Base), and of 5-HIAA, Under Conditions Described in Text.

Subject	Age (yr)	Condition	Serum phenylalanine (mg/100 ml)	Serotonin (μg/100 mg creatinine)	Tryptamine (μg/100 mg creatinine)	5-HIAA (μg/100 mg creatinine)
			Phenylketonurics			
5	16	Control	29.4	.4	4.7	65
		Tryptophan load*		0	13.1	95
		5-HTP load*		20.9	1.7	2,780
6	17	Control	35.0	0	2.2	70
		Tryptophan load*		0	12.2	105
		5-HTP load*		6.6	2.1	1,590
7	1	Low-phenylalanine diet	5.6	9.7	25.6	1,045
8	2	Low-phenylalanine diet	2.8	8.7	16.5	780
			Mongoloids			
9	16	Control		1.2	3.4	215
		Tryptophan load*		6.2	35.1	375
		5-HTP load*		25.9	7.8	3,190
10	16	Control		.2	5.5	235
		Tryptophan load*		4.3	38.9	355
		5-HTP load*		33.5	10.6	3,000
			Normal			
11	23	Control		1.6	2.4	175
		Tryptophan load*		12.0	46.2	560
		5-HTP load*		51.2	0	2,530

* Urine collected for 12 hr after amino acid loading.

urine of each of the 4 phenylketonurics on a normal diet. Even after administration of a large oral load of tryptophan, serotonin could not be detected in the urine. After administration of 5-HTP, the phenylketonuric subjects all excreted serotonin. By contrast, the 4 mongoloid subjects and the normal subject not only excreted serotonin during the control period, but showed a 2-fold or greater increase in urinary serotonin concentration after receiving a tryptophan load.

Urinary excretion of 5-HIAA, the major terminal metabolite of 5-HTP, paralleled the excretion of serotonin. The 4 phenylketonuric subjects excreted small amounts of 5-HIAA during the control period, but this was not significantly increased after administration of tryptophan. The 4 mongoloid subjects and the normal subject, on the other hand, displayed higher urinary 5-HIAA concentrations during the control period, as well as a significant increase in excretion of this metabolite after tryptophan loading. Urinary excretion of tryptamine was similar in all subjects, except that in the second experiment, where urine was collected for a shorter period after amino acid loading, excretion of tryptamine after administration of tryptophan was greater in the normal and mongoloid subjects than in the phenylketonurics.

When serum and tissue concentrations of phenylalanine had been reduced to normal levels in the 2 adult phenylketonurics by administration of a low-phenylalanine diet, small amounts of serotonin became detectable in the urine, and urinary excretion of 5-HIAA was clearly increased. The 2 phenylketonuric infants whose urine was collected while they were on a low-phenylalanine diet excreted relatively large amounts of serotonin comparable to amounts found in the urine of normal children in an earlier study(8). Their excretion of 5-HIAA exceeded that of the normal and mongoloid subjects, even after the latter had been administered a tryptophan load.

Discussion. Patients with mongolism were chosen as the controls for these experiments, since it seemed important to use readily diagnosed mental defectives from the same hospital environment for comparison with the phenylketonurics, to minimize possible variables related to intestinal flora, physical activity and environmental stress. Although an abnormality in tryptophan metabolism has been claimed in mongolism(11), a previous study by one of us(8) demonstrated no difference in urinary serotonin and tryptamine excretion between normal and mongoloid children.

The present study suggests that depression of aromatic L-amino acid decarboxylase by elevated tissue concentrations of phenylalanine may not be the only or even the chief reason for decreased serotonin production in untreated phenylketonuria. The mongoloid subjects showed greater urinary excretion of tryptamine than the phenylketonurics after an oral tryptophan load when urine was collected for 12 hours after the load, but there was little difference between the phenylketonuric and mongoloid subjects when urine was collected for 24 hours after tryptophan loading. Control tryptamine excretion by both groups of subjects was similar. The lower tryptamine excretion by the phenylketonurics in the first 12 hours after loading could be explained either by a decreased rate of decarboxylation, or by slower absorption of tryptophan from the gastro-intestinal tract.

Although it might be argued that some of the tryptamine found in urine results from decarboxylation of tryptophan by intestinal bacteria and subsequent absorption from the gut, urinary excretion of serotonin is a reliable measure of endogenous decarboxylation. In other experiments we have found that oral administration of large amounts of serotonin to normal subjects does not increase their excretion of this amine. The data in Tables I and II indicate that when 5-HTP is administered to untreated phenylketonurics, significant endogenous decarboxylation to serotonin does take place, although urinary excretion of serotonin tends to be lower than in mongoloids given 5-HTP.

On the other hand, the data show that the 5-hydroxylation of tryptophan is clearly depressed in phenylketonuria, possibly as a result of competitive inhibition by elevated tissue concentrations of phenylalanine. When

these are reduced to normal levels, the 5-hydroxylation of tryptophan is increased. The data also suggest that 5-HTP may normally be formed at a greater rate in infancy than in later life.

Tryptophan-5-hydroxylase was first demonstrated in the intestinal mucosa of rats and guinea pigs by Cooper and Melcer(12). Freedland *et al*(13,14) have shown that the phenylalanine hydroxylase enzyme system of mammalian liver also can convert tryptophan to 5-HTP, but the rate of hydroxylation of phenylalanine by this system is 30 times as great as that of tryptophan, and it seems unlikely that hepatic phenylalanine hydroxylase is physiologically significant in the production of 5-HTP(15). In any case, it is apparent that phenylketonurics do not have a genetically-determined inability to 5-hydroxylate troptophan, since this metabolic defect is corrected by a low-phenylalanine diet.

Several investigators(16-18) have recently demonstrated that brain serotonin concentrations are diminished in rats rendered artificially phenylketonuric by administration of large amounts of dietary phenylalanine. It has been shown that elevated concentrations of phenylalanine and certain other amino acids can interfere with the transport of tyrosine into rat brain both *in vitro* and *in vivo* (19-21). Recent studies also have demonstrated that high concentrations of phenylalanine and other amino acids interfere with the active transport of 5-HTP into rat brain both *in vitro* and in the living animal(22-24), and it is reasonable to speculate that a similar mechanism may be active in phenylketonuric man.

Several things are not known about the natural genetically-determined phenylketonuria of man. Is there a deficiency of serotonin in brain, and are tissue phenylalanine concentrations sufficiently high to decrease active transport of 5-HTP into brain? Since serotonin does not readily cross the blood-brain barrier and since tryptophan-5-hydroxylase has not been found in brain(12), it seems likely that tryptophan is converted to 5-HTP elsewhere in the body, is carried to the brain in blood, and after transport into brain is there decarboxylated to serotonin. What is the relative importance of defective 5-hydroxylation of tryptophan, and of impaired transport of 5-HTP into brain, in producing a serotonin deficiency in human phenylketonuric brain, if this does occur? Does a deficiency of serotonin in brain, especially during its period of rapid growth, contribute to the mental defect characteristic of phenylketonuria? Assays of serotonin concentration in autopsy specimens of human phenylketonuric brain, and studies of the effect of 5-HTP administration on the mental defect of artificially phenylketonuric monkeys(25) might solve these questions. It would seem reasonable to investigate the effect of supplementing the low-phenylalanine diet with 5-HTP in infants with phenylketonuria, since the low-phenylalanine diet by itself often fails to yield optimal intellectual development in children.

Summary. Untreated phenylketonuric patients excreted less serotonin and less 5-HIAA in their urine than did patients with mongolism. They also excreted less than a normal adult. The difference between phenylketonurics and the control subjects was accentuated after oral administration of tryptophan. When 5-HTP was given by mouth, the phenylketonuric subjects excreted relatively large amounts of serotonin. After their serum phenylalanine concentrations had been reduced to a normal level by the use of a low-phenylalanine diet, phenylketonurics spontaneously excreted normal amounts of serotonin and 5-HIAA. A reversible defect in the 5-hydroxylation of tryptophan thus seems primarily responsible for the decreased production of serotonin in phenylketonuria.

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Inhibition of Varicella Virus by 5-Iodo-2′-deoxyuridine. (28848)

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The demonstration that 5-iodo-2′-deoxyuridine (5-IDU) is a potent inhibitor *in vitro* of herpes simplex and vaccinia viruses(1) subsequently led to its successful use *in vivo* for treatment of keratitis of herpes simplex and vaccinia(2,3). Numerous publications have established that 5-IDU and certain related compounds inhibit only DNA-containing viruses. It has not been firmly established that varicella virus contains DNA exclusively; however, it does appear to share a number of characteristics with herpes viruses and usually is considered to be a member of this DNA-virus family(4).

The following studies were undertaken to determine if 5-IDU is a potent inhibitor of varicella virus, which would be indirect evidence that the virus contains DNA. Further, such a finding might suggest a potential use for this compound in selected individuals with chickenpox or herpes zoster.

Materials and methods. Tissue culture. Two strains of human diploid fibroblasts were used interchangeably, the one WI-38(5) was provided by Dr. Leonard Hayflick of Wistar Institute, and the other was derived from the chorionic villi of human placenta(6). Both strains were handled in the same manner(5), and no detectable differences between them were observed. Suspensions of trypsinized cells in Eagle's basal medium(7) with 10% fetal bovine serum and antibiotics (100 units of penicillin and 100 μg streptomycin per ml) were diluted to contain 1 to 2 $\times$ 10^5 cells/ml and 0.5 ml was planted in each culture tube. Tubes were incubated at 36°C in stationary racks slanted 5° until monolayers resulted (4 to 7 days). Then Eagle's medium was renewed but in this case it contained 0.01 M tris-buffer (pH 7.6).

Varicella virus. These studies involved a strain of varicella virus (Dr. E. Rosanoff, Wyeth Laboratories) originally isolated from a case of chickenpox and passed 24 times in human embryo kidney culture. It was maintained in this laboratory by continuous passage in human fibroblast cultures. Two additional strains of this virus, isolated from cases of chickenpox and herpes zoster, were also briefly studied. All virus strains were