stops on or about the 8th day(13) or approximately the point of irreversibility, and since interference with protein synthesis is a late step in theories of cataractogenesis, it is tempting to speculate that the point of irreversibility is coincident with the time when the restoration of protein synthesis is no longer possible.

Summary. Galactose-fed rats with unilateral cataracts develop cataracts in the second eye even though they are placed on a normal diet. Rats fed a 35% galactose diet for 14 days, followed by a normal diet, develop cataracts in a median time of 18 days just as if they were continuously on a galactose diet. The cataractogenic process becomes irreversible after 10-14 days of galactose. Restoration of a normal diet during the irreversible period results in a return of lens hydration, dulcitol and ATP to normal levels. It is suggested that changes such as these may be placed in the early or late period of cataractogenesis depending on whether they tend to return to normal or to continue their abnormal trend. Thus, the vacuoles seen after 2-4 days of galactose which are reported to be due to fiber swelling and bursting develop parallel with but are not the cause of the later white opaque cataracts.

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Studies on Inhibition of Brain 5-Hydroxytryptophan Decarboxylase by Phenylalanine Metabolites.* (29833)

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The conversion of 5-hydroxytryptophan (5HTP) to 5-hydroxytryptamine (5HT) is catalyzed by the enzyme 5-hydroxytryptophan decarboxylase (5HTPD)(1) and requires pyridoxal phosphate as a cofactor(2, 3). Davison and Sandler(4) have shown by *in vitro* studies that phenylpyruvic acid, phenyllactic acid, and phenylacetic acid inhibit 5HTPD. Huang and Hsia(5) have reported that this inhibition is competitive and substrate-dependent at pH 8 in rat kidneys.

The present communication describes experiments carried out to determine the inhibition of brain 5HTPD by phenylalanine derivatives.

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Materials and methods. For these studies, weanling guinea pigs were killed by decapitation, and a 20% homogenate of the brain was prepared. Brain 5HTPD was determined by the fluorometric method of McCaman and Robins(6). The final incubation mixture contained: 2-amino-2-methyl-1,3-propanediol buffer, 0.15 M, pH 8.05; bovine plasma albumin 0.05%; pyridoxal phosphate, 0.3 mM; isonicotinic acid-2-isopropylhydrazide (Hoffmann La Roche), 0.1 mM; DL-5-HTP (Sigma), 0.06-1.25 mM; and 0.25 ml homogenate in a total volume of 1.0 ml. Studies were performed in triplicate with 5 separate substrate concentrations. For the inhibition studies, 1.8×10^{-2}-5.4×10^{-2} M of phe-

nylalanine derivatives were dissolved in buffer and added to the incubation mixture prior to the start of the reaction. The mean values were plotted and eye-fitted curves were drawn for Lineweaver-Burk analysis(7).

Results. The kinetic data are summarized in Table I and Fig. 1. The K_m for 5HTPD in brain is 5.2×10^{-5} M. Of the phenylalanine derivatives, phenylpyruvic acid showed the greatest inhibition and p-hydroxyphenylacetic acid showed the least inhibition. In all instances, the inhibition of 5HTPD by phenylalanine derivatives is competitive and substrate-dependent at pH 8.05.

Discussion. Both Hanson(8) and Tashian (9) have reported the inhibition of brain L-glutamic acid decarboxylase by phenylalanine derivatives. The inhibition was found to be competitive, and at concentrations of 7.5×10^{-3} M, the inhibitor compounds were found to have a greater affinity than the substrate for the brain enzyme.

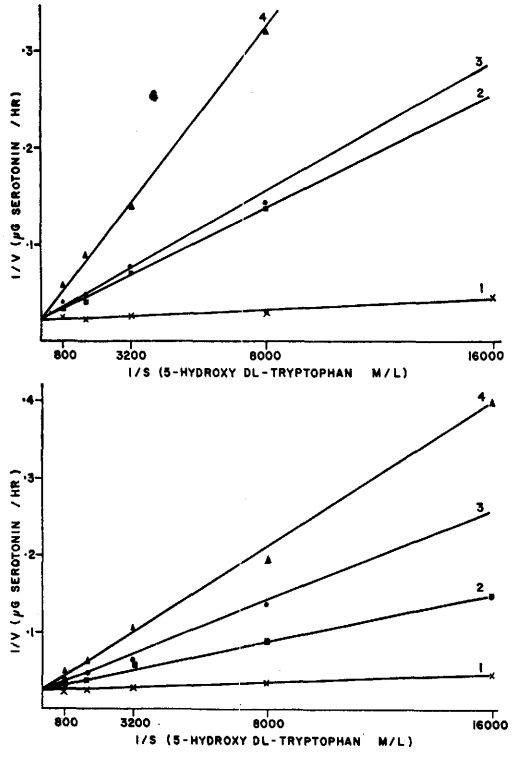

FIG. 1. A, top. Inhibition of 5-hydroxytryptophan decarboxylase in guinea pig brain homogenate. 1. Control, 2. Phenylacetic, 3. Phenyllactic, 4. Phenylpyruvic. B, bottom. Inhibition of 5-hydroxytryptophan decarboxylase in guinea pig brain homogenate. 1. Control, 2. p-hydroxyphenylacetic, 3. p-hydroxyphenyllactic, 4. p-hydroxyphenylpyruvic.

TABLE I. Inhibition of Brain 5HTPD by Phenylalanine Derivatives (expressed as $K_i \times 10^{-3}$).

Phenylpyruvic acid	0.63
Phenyllactic acid	4.5
Phenylacetic acid	2.5
p-hydroxyphenylpyruvic acid	3.0
p-hydroxyphenyllactic acid	5.2
p-hydroxyphenylacetic acid	10.4

In the present study, a similar mechanism may be seen with the inhibition of 5HTPD by phenylalanine derivatives. These observations can also be extended to include DOPA decarboxylase, tryptophan decarboxylase, and phenylalanine decarboxylase since Lovenberg *et al*(10) have shown that the decarboxylation of all the natural aromatic L-amino acids are catalyzed by a single enzyme system.

In phenylketonuria, the decrease of 5HT in the blood and 5-hydroxyindoleacetic acid in the urine has been attributed either to impaired hydroxylation of tryptophan or to an inhibition of 5HTPD by phenylalanine derivatives(11). In previous studies(5), it has been shown that concentrations of between 1.7×10^{-2} M of phenylalanine derivatives were required to bring about 50% inhibition of kidney 5HTPD *in vitro*, which is 10 times the level encountered in phenylketonuria. Recently, it has been suggested that the decrease of brain 5HT observed in experimental animals fed high phenylalanine diets might be the result of an inhibition of brain 5HTPD by phenylalanine derivatives (12). Direct assay of brain 5HTPD in such phenylketonuric animals failed to reveal any abnormalities(13,14). The present data would explain this since concentrations of 2.0×10^{-2} M of phenylalanine derivatives are required to bring about a 50% inhibition of brain 5HTPD, while the maximum concentrations of phenylalanine seen in serum are 3.3×10^{-3} M.

Thus, it would appear that the inhibition of 5HTPD by phenylalanine derivatives probably does not plan an important physiological role in the decrease of brain 5HT in experimental phenylketonuria, and that the excessive phenylalanine interferes either with the hydroxylation of tryptophan(15) or the active transport of 5HTP into brain(16).

Summary. The K_m for brain 5HTPD is 5.2×10^{-5} M. The inhibition of this enzyme by phenylalanine derivatives is competitive and substrate-dependent at pH 8.05. This inhibition does not appear to play a major role in the decrease of brain 5HT reported in experimental phenylketonuria.

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Relative Rates of Oxidation of Palmitate-1-C^{14} by White Blood Cells, Red Cells and Platelets of Rats.* (29834)

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Whole blood cells have been reported to incorporate acetate-C^{14} into lipids(1,2), to oxidize palmitate-1-C^{14} to $C^{14}O_2$(3) and to incorporate fatty acids into phosphatides, triglycerides and esters of cholesterol(4). Marks *et al*(5) have found that the rate of acetate-1-C^{14} incorporation into mixed lipids of normal white blood cells, on a per cell basis, averaged 80 times greater than that into the platelet lipids and at least 1000-fold the incorporation into lipids of red cells. The present investigation was done in order to provide comparable data on fatty acid oxidation. A preliminary report has appeared(6).

Methods. The blood of normal, fasting male rats, weighing between 300 and 400 g and killed by decapitation, was collected into an exact volume of isotonic medium (NaCl—0.137 M; KCl—0.004 M; Na_2HPO_4 buffered to pH 7.4—0.01 M), containing heparin (0.04 mg per ml) and then adjusted with the same medium in order to obtain exactly a 7.5 times diluted blood. About 50 ml of this diluted blood were centrifuged immediately at $1800 \times g$ for 30 minutes, to prepare cell-free plasma of the same dilution used to adjust the volumes of various cell populations separated from another aliquot part of the same diluted blood. Samples were also taken from this diluted blood for counts of white cells, platelets and red cells. Siliconized glassware was used throughout.

Separation of white blood cells, platelets and red cells. Various methods of separation of white blood cells, using fibrinogen(7), dextran(8), or other macromolecular substances such as polyvinylpyrrolidone(9) were tested. Many of these methods do not separate platelets, and very soon it was observed that dextran, fibrinogen, polyvinylpyrrolidone and other hydrophilic macromolecular substances, whether proteins (ovalbumin, beta globulin) or not (methyl cellulose, Elvanol polyvinyl

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