not appear to be related to that shown by 5-iodo-2-deoxyuridine (IDU). IDU acts as a thymidine antagonist and is infective against RNA containing viruses(10).

Summary. 3,5 diiodo-4-hydroxybenzenesulfonamide was found to inhibit the growth of the influenza virus in chick embryo lung tissue culture. The compound appeared to exert a selective direct effect upon the extracellular virus particle.

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Received March 9, 1964. P.S.E.B.M., 1964, v116.

Effects of Dietary Phenylalanine on Activity of Phenylalanine Hydroxylase from Rat Liver.* (29269)

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Conflicting reports exist concerning the effects of dietary phenylalanine and tyrosine on levels of phenylalanine hydroxylase from rat liver, especially as such effects are related to experimental phenylketonuria. Knox and Behrman concluded that a marked depression of the liver hydroxylase was demonstrated by feeding a diet high in tyrosine to young rats, but a diet also rich in phenylalanine does not affect the level of this enzyme(1). Contrary to this review, the data of Auerbach *et al,* whose work was cited, show a severe decrease in activity of phenylalanine hydroxylase in animals which received a diet to which 5% DL-phenylalanine was added as well as 2.5% each of DL-phenylalanine plus L-tyrosine or 5% L-tyrosine alone(2). These authors presumed that these amino acids cause a decreased production of the enzyme. To complicate matters further, a succeeding report by Freedland *et al* claimed a more moderate decrease of the hydroxylase occurs upon feeding L-phenylalanine, and that L-tyrosine feeding causes an increase in activity of the enzyme(3). Huang *et al* reported only a slight decrease in activity of the liver hydroxylase after feeding 3.75% L-tyrosine plus 2.75% DL-phenylalanine for up to 9 weeks. However, when only 2.5% DL-phenylalanine was added to the diet for 6½ or 9 weeks, no significant decrease in activity was noted(4). More recent investigations by Wang and Waisman show a 40 to 60% depression of phenylalanine hydroxylase levels in liver upon feeding L-phenylalanine to weanling male rats for 2 and 3 weeks(5). The depression elicited by the highest amount of phenylalanine fed (7%) was no greater than the lowest amount fed (3.5%), so that quantitation of the effect has not been adequately described. Moreover, no evidence has been presented which indicates whether decreases in measured activities of phenylalanine hydroxylase are real or apparent.

The present investigation was undertaken to clarify previous reports on the effects of dietary phenylalanine on activity of phenylalanine hydroxylase from rat liver, to quantitate more exactly the amount of phenylalanine necessary to produce a given lowering in hydroxylase activity, and to ascertain

* Supported by an N.I.H. Research Grant and by State University of New York.

TABLE I. Effects of Dietary L-Phenylalanine on Body Weight and Level of Liver Phenylalanine Hydroxylase from Rats.

Phenylalanine in diet, %	Wks on diet	Avg body wt, g*	Hydroxylase activity† Specific	Total
0	2	99.4 (5)	4.9 ± .9	14.8 ± 2.7
	3	146.4 (5)	4.1 ± .7	18.6 ± 7.2
1	2	109.0 (4)	3.7 ± .4	14.3 ± 3.2
	3	149.2 (5)	2.3 ± .9	12.2 ± 4.8
2	2	115.5 (4)	3.5 ± .4	11.2 ± 2.5
	3	147.0 (5)	2.2 ± .3	13.4 ± 2.4
3	2	113.0 (3)	2.8 ± .2	8.9 ± 3.1
	3	167.0 (5)	1.6 ± .6	9.9 ± 2.1
4	2	104.2 (4)	3.4 ± .4	7.6 ± 1.5
	3	166.8 (5)	1.4 ± .2	8.1 ± 3.0
5	2	86.2 (3)	2.2 ± .5	5.7 ± 3.2
	3	138.0 (3)	1.4 ± .4	8.6 ± 4.5

* Initial avg wt was 39.4 g with no. of rats per group shown in parentheses.
† Values represent avg with standard deviation.

whether the decrease in measured activity is real or apparent. A preliminary report of part of this work has been published(6).

Materials and methods. Male, 18-day-old, Sprague-Dawley rats† were fed *ad libitum* for 2 and 3 weeks on a ground commercial diet in which 0 to 5% L-phenylalanine at 1% increments was added. Pair-fed animals at 0 and 3.5% levels also were included. Food intakes were recorded at daily intervals. As an indication of growth, body weights were taken throughout the experimental periods.

Preparations and incubation mixtures for measuring activities of phenylalanine hydroxylase in supernatant solutions from liver homogenates were made according to Kenney *et al*(7) as modified by Freedland *et al*(8). Tyrosine formation was determined by the colorimetric method of Udenfriend and Cooper(9). Before assay, some liver supernatant solutions were dialyzed with stirring for 2 hours at 4° against 0.14 M KCl containing 0.01 M potassium phosphate buffer at pH 6.7. Protein was determined by the method of Lowry *et al*(10) with bovine serum albumin as standard. Specific activities of the hydroxylase are expressed as μmoles of tyrosine formed per hour at 37° per 100 mg of protein.

Results. The body weights and measured activities of phenylalanine hydroxylase from livers of rats which were fed 0 to 5% L-phenylalanine *ad libitum* for 2 or 3 weeks are given in Table I. The average gains in body weight on the moderate amounts of phenylalanine are better than those on low levels of this amino acid, but depression in growth occurs with phenylalanine added at the 5% level. Both specific and total activities are decreased significantly with increasing amounts of phenylalanine fed. Moreover, the feeding of as little as 1% of the L-isomer is sufficient to elicit a considerable depression of measured activity the relative degree of which is progressively less affected by increasing amounts of the amino acid fed. The decrease of hydroxylase activity after 3 weeks of feeding phenylalanine, especially at the higher levels, is greater than after 2 weeks.

The effects of a 2-hour dialysis on phenylalanine hydroxylase in supernatant solutions from livers of pair-fed rats which received 0 or 3.5% L-phenylalanine for 2 weeks are shown by the data in Table II. Pair-feeding does not alter the measured reduction of hydroxylase following dietary phenylalanine. Dialysis causes a decrease in measured hydroxylase from both control and experimental animals, and the relative loss of specific and total activities in the latter is as great as that seen in the former. These values show a 65% decrease in both specific and total activities after dialysis of hydroxylase preparations from rats which received no phenylalanine. The corresponding decrease is 67% after dialysis of preparations from rats which received 3.5% dietary phenylalanine. When compared with the control group, no apparent restoration of specific and total activities

TABLE II. Activities Before and After Dialysis of Liver Phenylalanine Hydroxylase from Rats.

Phenylalanine in diet, %	Avg body wt, g*	Dialysis	Hydroxylase activity† Specific	Total
0	125.8	—	4.6 ± .9	16.1 ± 4.9
		+	1.6 ± .5	5.6 ± 1.6
3.5	124.4	—	2.7 ±1.2	8.5 ± 1.8
		+	.9 ± .3	2.9 ± 1.1

* Ten animals in each group with avg initial wt of 52.9 g.
† Values represent avg with standard deviation.

† Holtzman Rat Co., Madison, Wisc.

by dialysis of hydroxylase preparations from the experimental group is then seen.

Discussion. There can no longer be any doubt as to the significant depression in apparent activity of liver phenylalanine hydroxylase from rats which are fed L-phenylalanine for 2 to 3 weeks. Even as little as 1% of this amino acid added to an adequate diet significantly lowers the measured activity of the enzyme. As no suppression of growth occurs with such a low level of phenylalanine in the diet, the decreased growth rates seen when large amounts are fed may be due to factors other than decreased hydroxylase alone.

Previous investigators, who have observed a decrease in measured phenylalanine hydroxylase following the feeding of tyrosine or phenylalanine, have tacitly assumed the decrease in enzyme to be real and not apparent without any experimental proof of either. Auerbach *et al*(2) suggested that their enzymatic results substantiate the hypothesis that feeding these aromatic amino acids inhibits the production of phenylalanine hydroxylase, but no evidence was given which excluded inhibition of the hydroxylase by accumulated catabolites which could conceivably lead to lowering of measured activity. The same authors also state that the decrease in phenylalanine hydroxylase due to a high phenylalanine diet may in part be explained by the marked inhibition of the activity of this enzyme by high levels of the amino acid as observed by Udenfriend and Cooper(11). This explanation is not valid, for the presence of significant phenylalanine in the enzyme preparation would allow considerable activity to be seen in the absence of additionally added substrate. Furthermore, if above optimal concentrations of substrate were in effect, addition of more phenylalanine should cause a decrease, not increase, in activity of the enzyme. Actually the data of Udenfriend and Cooper(11) show that inhibition of crude phenylalanine hydroxylase *in vitro* by amounts of phenylalanine which are several times above optimum causes only a moderate depression in activity. A decrease of approximately 30% can be calculated for a 10-fold excess of substrate.

The present results on the dialysis of preparations of phenylalanine hydroxylase from the livers of normal rats and from those fed an amount of L-phenylalanine sufficient to reduce considerably the measured level of enzyme over a 2-week period, would suggest that the decrease in hydroxylase in the latter case is due, at least in part, to a real suppression in synthesis of this enzyme rather than an apparent suppression of activity by accumulated catabolites of the amino acid. A hypothesis of catabolite inhibition must be experimentally tested when it is recalled that phenylalanine catabolites with modified side chains ending in carboxylate functions, *e.g.* phenylpyruvate, are effective competitive inhibitors of phenylalanine hydroxylase. As shown by Udenfriend and Cooper(11), even pyruvate, when added in a concentration which is equimolar to substrate phenylalanine, elicits an 85% inhibition of the enzyme. If such inhibitors were the primary cause of lower activity in hydroxylase preparations from rats fed phenylalanine, removal of these inhibitors by dialysis should result in less apparent loss in activity of these preparations than in dialyzed preparations from control animals. Since loss in activities upon dialysis of preparations from both experimental and control rats are approximately the same, such is not the case.

The condition of experimental phenylketonuria produced by feeding large amounts of phenylalanine to a normal animal may be attributable in some small part to the accumulation of toxic catabolites, but a decreased conversion of phenylalanine to tyrosine by lack of a normal level of hydroxylase is more likely. In the human defect of genetic origin, the actual lack of this enzyme has been well documented.

Summary. The activity of phenylalanine hydroxylase from livers of young, male rats fed L-phenylalanine is suppressed by as little as 1% of the amino acid fed for 2 weeks. Larger amounts and a longer period of feeding result in further decreases in measured activities whether animals are pair-fed or fed *ad libitum*. Growth is depressed also with high levels of phenylalanine in the diet. The lowered activity of the hydroxylase may be

attributable, at least partially, to a real suppression of enzyme synthesis.

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Received March 18, 1964. P.S.E.B.M., 1964, v116.

Inotropic Activity of a Series of Amino Acids.* (29270)

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The ability of the body to decarboxylate amino acids into more potent vasoactive amines has been indicated by previous studies(1,2,3). However, the initial reactants, that is, the amino acids, have generally been considered to be relatively inert circulatory agents(3). In this study a series of amino acids were examined in terms of their acute inotropic and pressor effects when administered in anesthetized, open-chest dogs.

Methods. Experiments were conducted in mongrel dogs of either sex, anesthetized with sodium pentobarbital, 30 mg/kg, i.v. After institution of interrupted positive pressure respiration, the thorax was opened in the midline and a strain gauge arch was sutured to the anterior aspect of the right ventricle for measurement of isometric systolic tension (4). Femoral arterial pressures were recorded from a polyethylene catheter attached to a Statham pressure transducer. Recordings were made on the Grass polygraph.

The amino acids, of highest commercial purity grade, were obtained from the Mann Research Laboratories. Except for l-arginine and l-cysteine, which were obtained as the monohydrochloride salt, the amino acids were obtained as the base. The acids were administered in Sorensen's phosphate or glycine buffer solution in volumes of 1 to 5 ml(5). In these vehicles, the amino acids were found to be relatively easily solubilized within certain specific pH ranges. These vehicles, in the pH ranges utilized in this study, failed to produce significant circulatory responses when injected into experimental animals. All doses are expressed as the base. Intravenous administration of the drugs was carried out by means of a polyethylene catheter placed in the femoral vein. Intra-aortic injections in a limited number of experiments were carried out by passing a catheter into the thoracic aorta by way of the femoral artery to the level of the diaphragm. This enabled relatively high concentrations of the amino acids to reach the adrenal glands and minimized the amount introduced into the general circulation(6). The drugs were flushed in with 5 cc of Ringer-Locke solution. Statistical analyses were carried out with the appropriate formulas(7).

Results. Individual amino acids generally produced biphasic or triphasic blood pressure responses, making it difficult to classify the responses into strict categories. By contrast,

* This research was supported by grants from South Carolina Heart Assn. and from Nat. Heart Inst., P.H.S.