A Paradoxical *in Vivo* Effect of L-Tryptophan on the Phosphoenolpyruvate Carboxykinase of Rat Liver*

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ABSTRACT: L-Tryptophan administered to intact or adrenalectomized rats greatly elevated phosphoenolpyruvate carboxykinase activity measured in the 105,000*g* (1 hr) supernatant fraction of liver, but *in vitro* addition of L-tryptophan had no effect on activity. Eighteen other L-amino acids given to intact rats caused little or no increase in enzyme activity. Administration of tryptophan did not alter the already high carboxykinase activity of alloxan diabetic rats. Of sixteen metabolic products or derivatives of tryptophan given to intact rats, only *N*-formyl-L-kynurenine, D-tryptophan, and DL-5-fluorotryptophan caused large increases in enzyme activity. The time course of the increase in carboxykinase after L-tryptophan was given to intact fasted rats showed a doubling of activity in the first hour and a slower rate of increase thereafter. Actinomycin D and puromycin inhibited 27 and 46%, respectively, the increase in activity during a period of 4 hr following administration of tryptophan. It is suggested that the rapid doubling of activity is a result of an activation process, but that both biosynthesis of carboxykinase and activation of newly synthesized enzyme contribute to further increases in activity.

L-Tryptophan blocked the glycogenic effect of hydrocortisone and prevented glycogenesis from pyruvate, malate, and aspartate, but not from glucose or glycerol. These data together with unpublished data on the hepatic concentration of various intermediates of gluconeogenesis indicate that *in vivo* catalysis by phosphoenolpyruvate carboxykinase is inhibited in rats given tryptophan.

Because glucagon produces a rapid elevation of hepatic phosphoenolpyruvate (PEP)[1] carboxykinase activity in the rat (Shrago *et al.*, 1963) and also causes an increase in the catabolism of amino acids (Izzo and Glasser, 1961), it was of interest to investigate the influence of administered amino acids on rat liver carboxykinase. A recent report from this laboratory (Young *et al.*, 1964) indicated that refeeding diets containing a high proportion of protein, but no carbohydrate, increased the already elevated levels of PEP carboxykinase in the livers of fasted rats. In the present study a remarkable increase in the activity of carboxykinase in the soluble fraction of liver homogenates occurred very rapidly when L-tryptophan was given to rats.

At least four other hepatic enzymes, tryptophan pyrrolase (Knox, 1951), tyrosine α-ketoglutarate transaminase (Kenney and Flora, 1961), and ornithine δ-transaminase and threonine dehydrase (Peraino *et al.*, 1965), are elevated following administration of tryptophan to rats. In the case of tryptophan pyrrolase, the increase in activity involves both activation of the apoenzyme by saturation with iron protoporphyrin (Greengard and Feigelson, 1961) and an increase in total enzyme protein (Feigelson and Greengard, 1962). The latter occurs mainly as a consequence of a decrease in the rate of degradation of the enzyme (Schimke *et al.*, 1964). The elevation of tyrosine transaminase activity by tryptophan is mediated apparently *via* biosynthesis of the enzyme (Rosen and Milholland, 1963).

In addition to reporting the experiments which led to the discovery of the *in vivo* elevation of PEP carboxykinase by tryptophan, this communication presents the results of preliminary studies on the mechanism underlying the increase in enzyme activity. The influence of various factors on the extent and duration of the response of carboxykinase to tryptophan and the effect of tryptophan administration on the formation of liver glycogen from several precursors were also investigated. The data indicate that administration of tryptophan elevates assayable PEP carboxykinase activity primarily by an activation process, but that *in vivo* catalysis by the enzyme is blocked.

Experimental Procedure

Normal and adrenalectomized male rats weighing from 140 to 180 g were obtained from the Badger Research Corp., Madison, Wis. The production of alloxan diabetes in rats and the care and use of all animals used in this work have been described (Foster *et al.*, 1966).

The following compounds were generous gifts from the indicated source: actinomycin D, Dr. N. Bohonos,

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[1] Abbreviation used in this work: PEP, phosphoenolypyruvate.

TABLE I: Hepatic PEP Carboxykinase Activity of Fed Rats 4 Hours after Intragastric Administration of L-Amino Acids.

Expt	Treatment[a] (no. of animals)	Activity (nmoles of PEP formed/min per mg of protein)
A	Control (2)	77.4
	+ Glutamic + arginine + histidine + lysine + proline (2)	86.6
	+ Aspartic + glycine + alanine (2)	75.3
	+ Cystine + cysteine + methionine (2)	82.6
	+ Serine + threonine + valine (2)	78.0
	+ Leucine + isoleucine + phenylalanine + tyrosine + tryptophan (2)	142
B	Control (2)	56.2
	+ Leucine (2)	54.3
	+ Isoleucine (2)	47.6
	+ Phenylalanine (2)	46.2
	+ Tyrosine (2)	45.1
	+ Tryptophan (2)	123

[a] Amino acids, singly or in combination (1 mmole of each amino acid/200-g rat), were given in 4–5 ml of aqueous solution or suspension.

Lederle Laboratories; puromycin dihydrochloride, Dr. D. DeLong, Eli Lilly Co.; and hydrocortisone, Drs. K. Folkers and M. Tishler, Merck and Co. Sources of purified L-amino acids and of various indole derivatives were as follows: proline, threonine, glutamic acid, phenylalanine, DL-5-hydroxytryptophan, D-tryptophan, DL-5-fluorotryptophan, and nicotinic acid, Nutritional Biochemicals Corp.; glycine, indole, 3-indolelactic acid, serotonin creatinine sulfate, reserpine, 5-hydroxyindole-3-acetic acid, DL-kynurenine, and tryptamine hydrochloride, Sigma Chemical Co.; tryptophan, cystine, and methionine, Mann Research Laboratories; lysine hydrochloride, tyrosine, leucine, anthranilic acid, 3-indoleacetic acid, and cysteine hydrochloride, Distillation Products; isoleucine, valine, and alanine, Hoffmann-LaRoche Inc.; indole-3-pyruvic acid, Aldrich Chemicals; aspartic acid, Fisher Scientific Co.; serine and *N*-formyl-L-kynurenine (B grade), Calbiochem; histidine hydrochloride, H M Chemical Co.; and arginine hydrochloride, General Biochemicals, Inc. Except for puromycin dihydrochloride which was purified by recrystallization (Fryth *et al.*, 1958), all chemicals were employed in the state of purity in which they were received.

Rats were killed by decapitation and allowed to bleed freely before excision of the liver; the latter was quickly weighed and then stored temporarily in 0.25 M sucrose in a beaker embedded in cracked ice. Livers were homogenized at 1–2° in 9 ml of 0.25 M sucrose/g of tissue and the homogenates were centrifuged at 105,000*g* and at 0–1° for 1 hr. The supernatant fractions thus obtained were assayed immediately for their PEP carboxykinase activity since the preparations from animals treated with tryptophan lost considerable activity if stored overnight at −10°. The assay described by Nordlie and Lardy (1963) was employed except that KCl and sucrose were omitted and PEP was cleaved with mercuric ion (Lohmann and Meyerhof, 1934); the liberated inorganic phosphate was estimated by the method of Sumner (1944). Care was taken that the amount of supernatant fraction assayed be small enough to ensure a zero-order reaction throughout the period of incubation.

Procedures for the determination of blood glucose and hepatic glycogen concentrations and for estimation of protein in the high-speed supernatant fraction of rat liver have been described (Foster *et al.*, 1966). The doses of the various compounds given to rats in this work, the route of their administration, and the vehicles are indicated in the tables and figures. Control rats received doses of the appropriate vehicles. Reagents for assays and solutions or suspensions for administration to rats were prepared with deionized, glass-distilled water.

Results

Influence of Amino Acids on Liver PEP Carboxykinase. In the initial experiment in which the influence of amino acids on hepatic PEP carboxykinase was studied, nineteen amino acids divided into five groups were given by stomach tube to fed rats (Table I, expt A). For unknown reasons, the basal carboxykinase activity of the rats used in this experiment was about 20 units higher than normal. Administration of the group of amino acids which consisted of leucine, isoleucine, phenylalanine, tyrosine, and tryptophan resulted in a 2-fold elevation of carboxykinase activity within 4 hr. When these five amino acids were given individually, only tryptophan caused an increase in carboxykinase activity (Table I, expt B). The possibility that some amino acids failed to elevate carboxykinase when administered

TABLE II: Hepatic PEP Carboxykinase Activity of 24-Hour Fasted Rats 4 Hours after Intragastric Administration of Combinations of L-Amino Acids with or without L-Tryptophan.

Treatment[a] (no. of animals)	Activity ± Std Dev (nmoles of PEP formed/min per mg of protein)
Control (4)	103 ± 5
+ Glutamic + arginine + histidine + lysine + proline (group A) (5)	142 ± 11
+ Aspartic + glycine + alanine + cystine (group B) (5)	122 ± 6
+ Methionine + serine + threonine + valine (group C) (5)	132 ± 3
+ Leucine + isoleucine + phenylalanine + tyrosine (group D) (5)	111 ± 6
+ Group A + tryptophan (5)	319 ± 12
+ Group B + tryptophan (5)	342 ± 36
+ Group C + tryptophan (5)	339 ± 19
+ Group D + tryptophan (5)	361 ± 32
+ Tryptophan (4)	320 ± 24

[a] Each combination of amino acids (1 mmole of each amino acid/200-g rat) was given in 3–4 ml of aqueous solution or suspension.

TABLE III: The Effect of L-Tryptophan Administered by Different Routes on the Hepatic PEP Carboxykinase Activity of Intact, Adrenalectomized, and Diabetic Rats.

Expt	Treatment	Activity ± Std Dev[a] (nmoles of PEP formed/min per mg of protein)		
		Intragastric	Subcutaneous	Intraperitoneal
A	Normal	56.0 ± 6.2 (3)	—	56.5 ± 6.8 (3)
	+ Tryptophan[b]	128 ± 33 (4)	—	164 ± 13 (6)
B	24-hr Fasted Normal	107 (2)	—	108 ± 5 (6)
	+ Tryptophan[b]	307 ± 14 (5)	309 ± 19 (6)	296 ± 27 (10)
C	Adrenalectomized	44.6 ± 3.4 (3)	—	46.3 ± 3.2 (3)
	+ Tryptophan[b]	128 (2)	—	122 ± 15 (5)
D	24-hr Fasted Adrenalectomized	—	—	107 ± 3 (3)
	+ Tryptophan[b]	—	—	265 ± 14 (5)
E	Alloxan diabetic	—	—	330 ± 26 (4)
	+ Tryptophan[b]	—	—	303 ± 60 (4)
	+ Tryptophan (12 hr before death)[c]	—	—	299 (2)

[a] Number of animals is given in parentheses. [b] Tryptophan, 75 mg/100-g rat, was given in aqueous suspension if by stomach tube or in 0.9% NaCl if subcutaneously or intraperitoneally. Animals were killed 4 hr after treatment. [c] Dose of tryptophan: 225 mg/100 g.

intragastrically to the fed rat because their absorption was hindered by the presence of food in the gastrointestinal tract was considered; therefore, 24-hr fasted rats were used to reexamine the influence of amino acids on carboxykinase. Table II gives the results of experiments in which four different groups of amino acids, each group with and without tryptophan, were administered to fasted rats. Those combinations of amino acids which did not include tryptophan caused 8–38% increases in hepatic carboxykinase activity. When tryptophan was included in each combination, increases in activity of 210–250% occurred. Tryptophan alone elevated activity by 211%. Tryptophan, when present at concentrations as high as 1.7×10^{-3} M, had no effect on the activity of PEP carboxykinase *in vitro*.

The influence of route of administration of tryptophan on the extent of elevation of carboxykinase was investigated (Table III). In intact fed rats (expt A) intraperitoneal administration of tryptophan resulted in slightly greater elevation of the enzyme than did intragastric administration. There was, however, no difference in the extent of elevation of carboxykinase in

TABLE IV: The Influence of Combinations of Hydrocortisone, Glucose, and L-Tryptophan on the Hepatic PEP Carboxykinase Activity, Blood Glucose, and Liver Glycogen of 24-Hour Fasted Rats.

Treatment[a] (no. of animals)	Activity ± Std Dev[b] (nmoles of PEP formed/min per mg of protein)	Blood Glucose (mg %)	Liver Glycogen (g %)
Control (3)	112 ± 2 +	76 ± 7	0.57 ± 0.25
+ Hydrocortisone (3)	138 ± 7	98 ± 6	1.41 ± 0.11
+ Glucose (3)	80 ± 3	83 ± 3	2.59 ± 0.72
+ Tryptophan (3)	299 ± 19 ++	73 ± 9	0.01 ± 0.01
+ Hydrocortisone + glucose (3)	98 ± 8 +	111 ± 5	3.54 ± 0.38
+ Glucose + tryptophan (3)	212 ± 11	117 ± 7	3.16 ± 1.00
+ Hydrocortisone + tryptophan (3)	299 ± 7 ++	78 ± 5	0.01 ± 0.02
+ Hydrocortisone + glucose + tryptophan (3)	237 ± 9	111 ± 8	3.01 ± 0.87

[a] Doses: hydrocortisone, 25 mg/kg, subcutaneously in 0.9% NaCl; glucose, 5 g/kg, intragastrically in water; tryptophan, 75 mg/100 g, intraperitoneally in 0.9% NaCl. All compounds were given 4 hr before the animals were killed. [b] Values marked with the same sign (+ or ++) are not significantly different ($P > 0.05$). Significance of differences between sample means was tested by Duncan's New Multiple Range Test (Steel and Torrie, 1960).

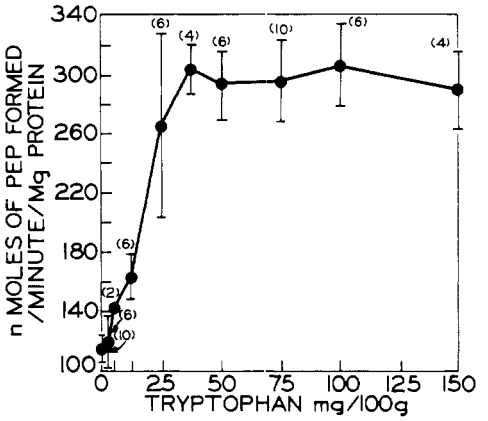

FIGURE 1: Hepatic PEP carboxykinase activity of 24-hr fasted rats 4 hr after intraperitoneal administration of increasing amounts of L-tryptophan in 0.9% NaCl. The number of animals contributing to each point is given above the bars which represent standard deviations.

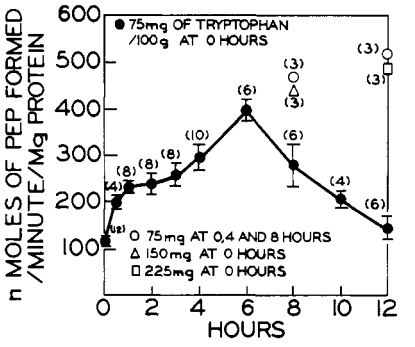

FIGURE 2: Changes in hepatic PEP carboxykinase activity of 24-hr fasted rats killed 0–12 hr after intraperitoneal administration of L-tryptophan in 0.9% NaCl. The number of animals contributing to each point is given in brackets. The bars represent standard deviations.

intact fasted rats (expt B) when tryptophan was given intragastrically, subcutaneously, or intraperitoneally. In succeeding experiments tryptophan was given intraperitoneally.

The relation between amount of tryptophan administered and the enhancement of hepatic PEP carboxykinase of intact fasted rats killed 4 hr after treatment is illustrated in Figure 1. As little as 5 mg/100 g caused a detectable increase in activity. The elevation of activity was maximum when the dose of tryptophan was 38 mg/100 g. In most of the following work a dose of 75 mg/100 g was given to ensure consistent effects.

Time Response to Tryptophan. Figure 2 shows the changes in hepatic carboxykinase activity that occurred in intact fasted rats during a period of 12 hr after administration of tryptophan. There was a striking elevation of the enzyme within 0.5 hr, and by 1 hr activity was elevated about 2-fold. Between 1 and 6 hr activity increased at a slower rate than in the first hour. When the dose of tryptophan was 75 mg/100 g, activity reached a peak at 6 hr and thereafter declined. If 75 mg was given at the beginning of the experiment and again at intervals of 4 hr., activity increased nearly 5-fold in 12 hr. The administration of a 150- or 225-mg dose of tryptophan at zero hours resulted in nearly the same elevation of activity at 8 and 12 hr, respectively, as when doses of 75 mg were given at intervals of 4 hr.

Hormonal and Metabolic Influences on the Response to Tryptophan. The effect of tryptophan on the hepatic PEP carboxykinase activity of adrenalectomized and alloxan diabetic rats is presented in Table III. Trypto-

phan elevated the carboxykinase of both fed (expt C) and fasted (expt D) adrenalectomized rats, but the increase was significantly less than that in intact animals. The initially high carboxykinase activity in the livers of diabetic rats was not altered by tryptophan (expt E).

The influence of hydrocortisone and glucose on the elevation of PEP carboxykinase by tryptophan was investigated (Table IV). In the fed or fasted rat glucose suppresses and hydrocortisone enhances carboxykinase activity (Shrago *et al.*, 1963; Young *et al*, 1964; Foster *et al.*, 1966). These effects of glucose and hydrocortisone were also observed in the present study, but hydrocortisone did not augment the response to tryptophan. The administration of glucose reduced the response of the enzyme to tryptophan by about 30%. Hydrocortisone tended to reverse this effect of glucose.

In an effort to establish whether the increase in PEP carboxykinase after administration of tryptophan was mediated by an influence of the amino acid on the rate of biosynthesis of either m-RNA or enzyme protein, actinomycin D and puromycin were employed. When given in sufficient doses, actinomycin D (Trakatellis *et al.*, 1964) and puromycin (Gorski *et al.*, 1961) are very effective inhibitors of the *in vivo* synthesis of m-RNA and protein, respectively. In animals given tryptophan 4 hr before death, actinomycin D caused 27% inhibition and puromycin 46% inhibition of the increase in activity which occurred when tryptophan alone was given (Table V).

Structural Requirements for Response by PEP Carboxykinase. Various indole derivatives which are intermediates, products, or inhibitors of tryptophan metabolism were tested for their ability to alter PEP carboxykinase activity in the livers of intact fasted rats (Table VI). Most of the compounds were administered in doses greater than the minimum dose of L-tryptophan (0.37 mmole/200 g) required for maximum elevation of carboxykinase activity in 4 hr (Figure 1). Under these conditions *N*-formyl-L-kynurenine, D-tryptophan, and DL-5-fluorotryptophan were, respectively, 87, 73, and 48% as effective as L-tryptophan in increasing activity above control levels. The other compounds tested were less than 25% as active as L-tryptophan. When the activity of *N*-formyl-L-kynurenine was compared with that of a suboptimal dose of L-tryptophan, the former was only 48% as effective as the latter (Table VI, bot-

TABLE V: The Influence of Actinomycin D and Puromycin on the Hepatic PEP Carboxykinase Activity of 24-Hour Fasted Rats Given L-Tryptophan 4 Hours before Death.

Treatment[a] (no. of animals)	Activity ± Std Dev (nmoles of PEP formed/min per mg of protein)
Control (8)	117 ± 12
+ Tryptophan (10)	296 ± 27
+ Actinomycin D (9)	91 ± 12
+ Actinomycin D + tryptophan (6)	248 ± 24
+ Puromycin (6)	108 ± 8
+ Puromycin + tryptophan (5)	214 ± 13

[a] Doses: tryptophan, 75 mg/100-g rat, intraperitoneally in 0.9% NaCl; actinomycin D, 875 μg/kg, intraperitoneally in 0.9% NaCl 0.5 hr before tryptophan; puromycin dihydrochloride was dissolved in water, adjusted to pH 6.5 with sodium hydroxide, and injected intraperitoneally in doses of 87 mg/kg given at hourly intervals beginning 0.5 hr before tryptophan.

TABLE VI: The Effect of Various Indole Derivatives on the Hepatic PEP Carboxykinase Activity of 24-Hour Fasted Rats.

Compd Administered[a] (no. of animals)	Dose (mmoles/200 g)	Activity ± Std Dev (nmoles of PEP formed/min per mg of protein)
None (6)	—	108 ± 5
L-Tryptophan (6)	0.5	294 ± 23
N-Formyl-L-kynurenine (1)	0.5	270
D-Tryptophan (4)	0.74	244 ± 13
DL-5-Fluorotryptophan (4)	0.5	197 ± 15
DL-Kynurenine (3)	0.5	151 ± 14
Nicotinic acid (4)	0.5	143 ± 12
3-Indolelactic acid (4)	0.5	141 ± 33
Indole-3-pyruvic acid (2)	0.5	142
Indole-3-acetic acid (2)	0.5	136
5-Hydroxyindole-3-acetic acid (2)	0.5	116
DL-5-Hydroxytryptophan (1)	0.5	115
Tryptamine·HCl (2)	0.05	117
5-Hydroxyindoleacetic acid (2)	0.5	116
Serotonin creatinine sulfate (3)	0.05	132 ± 9
Anthranilic acid (2)	0.5	115
Indole (4)	0.5	106 ± 6
Reserpine (2)	0.1	135
None (2)	—	141
L-Tryptophan (3)	0.1[b]	232 ± 7
N-Formyl-L-kynurenine (3)	0.1[b]	185 ± 8

[a] All compounds were given intraperitoneally in 0.9% NaCl and, if necessary, the pH was adjusted to 6.5–7. Except as indicated by footnote *b*, the compounds were administered 4 hr before the animals were killed. [b] Animals were killed 1 hr after treatment.

tom). These data are shown in a separate part of the table because the control rats used in this experiment had a carboxykinase activity much higher than normal. Neither *N*-formyl-L-kynurenine, D-tryptophan, or DL-5-fluorotryptophan, when present at concentrations as high as 3×10^{-3} M, had any effect on the activity of PEP carboxykinase *in vitro*.

The effect of tryptophan on the in vivo formation of liver glycogen from several glycogen precursors is given in Table VII. In fasted rats not given a precursor of glycogen, tryptophan caused the disappearance of the little glycogen initially present; however, this apparent glycogenolytic effect of tryptophan was not encountered when glucose and glycerol were given, for tryptophan did not severely diminish the formation of hepatic glycogen from these precursors. In contrast to the results obtained with glucose and glycerol, the formation of liver glycogen from pyruvate, malate, or aspartate was completely prevented by treatment of the animals with tryptophan.

TABLE VII: The Influence of L-Tryptophan on the Formation of Hepatic Glycogen from Glycogen Precursors Administered to 24-Hour Fasted Rats.

Precursor[a] (no. of animals)	Hepatic Glycogen Concentration ± Std Dev (g %)	
	− Tryptophan	+ Tryptophan[b]
None (9)	0.38 ± 0.23	0.00
Glucose (3)	2.57 ± 0.72	3.16 ± 1.00
Glycerol (4)	4.01 ± 0.40	2.95 ± 0.28
Pyruvate (2)	2.89	0.00
Malate (3)	1.19 ± 0.21	0.00
Asparate (3)	0.71 ± 0.32	0.01 ± 0.01

[a] Each precursor, 5g/kg, was given in 4–5 ml of aqueous solution by stomach tube 4 hr before death. Pyruvate, malate, and asparate were given as their sodium salts. [b] Tryptophan, 75 mg/100 g, was given intraperitoneally in 0.9% NaCl.

Discussion

It is apparent from Tables I and II that among the L-amino acids tryptophan is unique in the extent to which its administration to rats elevates PEP carboxykinase activity in the soluble fraction of liver homogenate. Since elevation of the enzyme by the other amino acids which were given to intact rats did not exceed 38%, and since hydrocortisone elevated activity by about this amount (Table IV), it may be that minor increases in activity after administration of relatively large doses of amino acids represent an adrenal-mediated response. This hypothesis has not been tested experimentally, but it is supported by the observation that in adrenalectomized rats the response of the enzyme to tryptophan was about 20% less than in intact rats (Table III).

The results of studies in which actinomycin D and puromycin were given to rats in an attempt to block elevation of carboxykinase in response to tryptophan administration (Table V) indicate that a major part of the increase in activity cannot be attributed to synthesis of new enzyme. Actinomycin D at the dose employed would have severely inhibited an increase in enzyme biosynthesis mediated *via* an effect of tryptophan on the rate of formation of m-RNA, but in fact it inhibited the tryptophan-induced increase in activity only 27%. Puromycin was given in doses sufficient to inhibit almost completely an increase in carboxykinase activity mediated *via* enzyme biosynthesis; it inhibited the response of carboxykinase to tryptophan only 46%. It is apparent, nevertheless, that biosynthesis of carboxykinase is necessary to get maximum elevation of activity by tryptophan.

The time course of the elevation of PEP carboxykinase after administration of tryptophan (Figure 2) is also taken as evidence that a major part of the tryptophan-induced increase in activity does not represent an increase in enzyme concentration. The data show a 74% increase in activity within 0.5 hr and a 100% increase within 1 hr after injection of tryptophan. When sufficient tryptophan was given to ensure maximum stimulation of carboxykinase for 12 hr, the average rate of increase in activity between the first and twelfth hour was only 25% of the rate during the first hour. The abrupt change in the slope of the curve at the 1-hr point indicates that elevation of assayable carboxykinase after administration of tryptophan occurs in two phases; the mechanism underlying the increase in activity may be different for each phase. The rapidity of the early response suggests an activation process such as conversion of an inactive form of the enzyme to an active form or alteration of the enzyme to a form with intrinsically greater activity. Although the rate of increase in activity during the period from 1 to 12 hr after injection of tryptophan was much less than the rate during the first hour, *it was two to three times greater than the maximum rate of increase observed after treatment of rats with hydrocortisone* (Foster *et al.*, 1966). The increase in carboxykinase following hydrocortisone administration is believed to be due to an increase in the concentration of the enzyme mediated *via* enzyme synthesis. In the case of tryptophan administration, it is suggested that after the initial burst of activity both biosynthesis of carboxykinase and activation of the newly synthesized enzyme contribute substantially to the further increase in carboxykinase activity.

By studying the kinetics of the elevation of tryptophan pyrrolase in rats treated with hydrocortisone and tryptophan, Schimke *et al.* (1964) were able to conclude that tryptophan protects tryptophan pyrrolase from *in vivo* degradation. Sufficient data has not yet been obtained on the kinetics of the tryptophan-induced increases in PEP carboxykinase to say whether tryptophan may also stabilize this enzyme; however, the observations on the effect of administered glucose on elevation of carboxykinase by tryptophan (Table IV) may be informative.

Glucose was much more effective than puromycin in depressing the elevated carboxykinase activity of fasted rats (Foster *et al.*, 1966), and it was suggested that glucose might be acting not only by inhibiting synthesis of carboxykinase but also by accelerating the rate of enzyme degradation. Since inhibition by glucose of the tryptophan-induced response of carboxykinase was no greater than inhibition by puromycin (Table V), it may be that tryptophan also stabilizes PEP carboxykinase.

The administration of tryptophan to alloxan diabetic rats did not alter the already high activity of hepatic PEP carboxykinase even when a dose of 225 mg/100 g was given 12 hr prior to death (Table III). This dose of tryptophan given to intact fasted rats for the same period (Figure 2) elevated activity to a value much higher than that of control, alloxan diabetic rats (Table III). These data may indicate that elevation of carboxykinase in diabetes is largely due to an activation process like that believed to occur when tryptophan is given to nondiabetic animals, but that some factor of the diabetic state imposes a maximum on the extent to which carboxykinase activity can be elevated.

Rosen and Milholland (1963) found that several compounds containing the indole nucleus elevate both tyrosine transaminase and tryptophan pyrrolase in livers of intact and adrenalectomized rats; the response of these enzymes was generally greater in the intact animal. In the present investigation seventeen derivatives of indole, including L-tryptophan, were tested for their ability to elevate carboxykinase in the livers of intact fasted rats (Table VI). Only L-tryptophan, *N*-formyl-L-kynurenine, D-tryptophan, and DL-5-fluorotryptophan caused increases in carboxykinase activity which were of sufficient magnitude to make it unlikely that the elevation was due to an adrenal-mediated response (*cf.* elevation by hydrocortisone, Table IV). Since the *N*-formyl-L-kynurenine used was not of purity comparable to that of other compounds tested, its apparent ability to elevate carboxykinase will be reinvestigated when a purer preparation can be made. Schayer (1950) demonstrated with the aid of an isotopic label in the indole ring that the rat readily converts D-tryptophan to L-tryptophan. The compound active in elevating PEP carboxykinase, if not L-tryptophan itself, must be very closely related to tryptophan in its structure.

Although the administration of tryptophan to rats results in a striking elevation of the activity of PEP carboxykinase measured in the soluble fraction of liver homogenates, such treatment also causes inhibition of glycogenesis from precursors beyond PEP carboxykinase in the gluconeogenic pathway (Table VII). Furthermore, the glycogenic effect of hydrocortisone is prevented by administration of tryptophan (Table IV). Rosen and Nichol (1964) reported that tryptophan blocked the glycogenic activity of cortisol and depressed the level of glycogen in livers of fed or fasted rats. These authors suggested that tryptophan has a glycogenolytic activity which masks the glycogenic activity of cortisol. The data of Table VII show, however, that tryptophan does not greatly affect glycogenesis from glucose or glycerol. The apparent glycogenolytic effect of tryptophan is due presumably to mobilization of hepatic glycogen reserves in order to meet requirements for glucose normally met by gluconeogenesis. The inhibitory effect of tryptophan on gluconeogenesis has been investigated, and it appears to be a result of blocking of the catalytic function of PEP carboxykinase *in vivo*.[2]

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[2] A paper to be published (Ray, Foster, and Lardy) will present data which show that in animals treated with trypotophan the malate, lactate, and pyruvate concentrations in the liver are greatly increased over normal levels and that PEP and other gluconeogenic intermediates are greatly decreased.