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EFFECTS OF DRUGS ON THE METABOLISM OF TRYPTOPHAN.

ALPHA-HYDRAZINOTRYPTOPHAN AND OTHER AMINO ACID ANALOGS*

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Abstract—The formation of respiratory $^{14}CO_2$ from injected tryptophan-^{14}C is reduced *in vivo* after the simultaneous administration of certain analogs of tryptophan. α-Hydrazinotryptophan lowered the rate of metabolism of tryptophan-2-^{14}C (label in the pyrrole ring) and, to an even greater extent, that of tryptophan-3′-^{14}C (label in the side chain). The oxidation of tryptophan-benzene-^{14}C was unaffected by this analog. α-Hydrazino-5-hydroxytryptophan had the same relative action on the metabolism of tryptophan-2- and -3′-^{14}C, but the effect was smaller. Several compounds that diminished the rate of oxidation of tryptophan *in vivo* inhibited tryptophan pyrrolase *in vitro*. A notable exception is tryptophol, which inhibited tryptophan pyrrolase by 92 per cent when present at a concentration of 10^{-3} M *in vitro*, but which gave inconclusive results *in vivo*. The pattern of inhibition of the breakdown of differentially labeled metabolites provides a useful tool in detecting rate-limiting steps in a metabolic sequence *in vivo*.

ON THE BASIS of the antimetabolite hypothesis, investigators have assessed the effects of indole alkaloids on the action and metabolism of serotonin.[1–5] This point of view has led to the search for substances, whether analogs of serotonin or not, that would modify the biological activity of this compound in the brain and other organs. A valuable product of this research has been the discovery of compounds like α-propylphenylacetamide,[6–9] *p*-chlorophenylalanine,[9] the chlorinated amphetamines,[10, 11] and other types of ring-substituted amphetamines[6, 12] that inhibit tryptophan hydroxylase and can lead to an actual reduction in the concentration of serotonin *in vivo*.

Although the kynurenine pathway of tryptophan metabolism is regarded as the main one regulating tissue concentrations of tryptophan, there are few studies of the effect of drugs upon this route. Most of these studies are based upon experiments carried out *in vitro*, and they have been concerned especially with the induction and inhibition of tryptophan pyrrolase. Among the inhibitors that have been described for this enzyme are α-methyltryptophan[13, 14] 5-hydroxytryptophan,[15, 16] tryptazan,[17] 5-methyltryptophan,[17] catecholamines,[15] 3-hydroxyanthranilic acid[18] and other metabolites of tryptophan,[15, 18] and estrogens.[19]

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The main aim of the present work was to investigate the action of tryptophan analogs and related compounds on the metabolism of tracer doses of ^{14}C-tryptophan to respiratory $^{14}CO_2$ in the rat. In order to explore these actions further, some experiments *in vitro* were also carried out.

EXPERIMENTAL

The metabolism of tryptophan *in vivo* was studied as previously described for tyrosine[20] and tryptophan[20–22] by using samples of tryptophan that were variously labeled: DL-tryptophan-3′-^{14}C (sp. act., 4·08 to 10·3 mc/m-mole in various lots) and L-alanine-U-^{14}C (sp. act., 111 mc/m-mole) from New England Nuclear Corp., Boston, Mass.; DL-tryptophan-2-^{14}C (sp. act., 1·08 mc/m-mole) from Nuclear Research Chemicals, Orlando, Fla.; DL-tryptophan-^{14}C, uniformly labeled in the benzene ring (sp. act., 3·81 mc/m-mole) from Nuclear-Chicago, Des Plaines, Ill.; and sodium formate (sp. act., 13 mc/m-mole) from Merck Sharp & Dohme Ltd., Montreal.

The following compounds were gifts, as indicated, and are gratefully acknowledged: α-hydrazino-β-(3-indolyl)propionic acid (α-hydrazinotryptophan), α-hydrazino-β-(5-hydroxyindolyl)propionic acid (α-hydrazino-5-hydroxytryptophan), α-methyl-5-hydroxytryptophan, α-methyltyrosine, α-methyl-*m*-tyrosine, α-methyl-*m*-methoxyphenylalanine, α-methyl-3,4-dimethoxyphenylalanine, α-methyl-α-hydrazino-β-(3,4-dihydroxyphenyl)propionic acid, L-α-methyldopa and hypaphorine hydrochloride from Merck Sharp & Dohme Research Laboratories, Rahway, N.J.; α-methyltryptamine, α-ethyltryptamine acetate, *N*-acetyltryptamine, β-indolylethanol (tryptophol), and 4-hydroxytryptophan from the Upjohn Company, Kalamazoo, Mich. The following compounds were purchased: L-tryptophan, 5-methyltryptophan and 6-methyltryptophan (Mann Research Laboratories, Inc., N.Y.); D-tryptophan, tryptamine hydrochloride and 5-hydroxytryptamine creatinine sulfate (California Corp. for Biochemical Research, Los Angeles); 5-hydroxytryptophan (Cyclo Chemical Corp., Los Angeles); *N*,*N*-dimethyltryptamine bioxalate and *N*,*N*-diethyltryptamine bioxalate (Chemical Procurement Laboratories, Inc., College Point, N.Y.); indole-3-acetic acid (Brickman and Co., Montreal); and dihydrazine sulfate (Mathieson Chemical Corp., Baltimore, Md.). All compounds were racemic mixtures except where otherwise noted.

Rats of the Sprague–Dawley strain, weighing 100–150 g, were used. The source of tryptophan pyrrolase for experiments *in vitro* was the liver, which was homogenized (25%, w/v) in 0·14 M KCl, containing 0·005 N NaOH. The enzyme was assayed by a modification[23] of the method of Knox and Auerbach.[24] Animals were adrenalectomized 4–7 days before use, in order to avoid increases in pyrrolase activity caused by release of corticosterone in response to stress.[17]

RESULTS

Effect of tryptophan analogs and derivatives in vivo. Various analogs and derivatives of tryptophan were tested in adrenalectomized rats for their action on the conversion of DL-tryptophan-3′-^{14}C to $^{14}CO_2$. The effects are set out in Table 1, calculated for a 2-hr period ("early effects") and a 6-hr period ("plateau effects"; see ref. 21). Of the compounds tested, only the D-isomer of tryptophan and α-methyl-5-hydroxytryptophan accelerated metabolism of the labeled tryptophan *in vivo*. It is known that D-tryptophan is one of the very few compounds that evokes an induction of tryptophan pyrrolase *in vivo*,[17] presumably by its conversion to the L-isomer

through oxidative deamination and asymmetric trans(re)amination. In three experiments the accelerating effect of D-tryptophan was variable, but in any given experiment it was constant through the 6-hr period. In a comparison with the effect of L- and D-tryptophan, the former proved considerably more effective, and its accelerating effect on the catabolism of the labeled tracer dose of tryptophan increased with time.

TABLE 1. EFFECT OF TRYPTOPHAN ANALOGS ON THE RATE OF OXIDATION OF TRYPTOPHAN-3′-^{14}C TO $^{14}CO_2$ IN ADRENALECTOMIZED RATS*

Compound	Dose (mg/kg)	Per cent of control at 2 hr	Per cent of control at 6 hr
L-Tryptophan	200	260	580
D-Tryptophan	200	170	240
D-Tryptophan	200	315	313
D-Tryptophan	200	407	316
5-Methyltryptophan	200	100	74
6-Methyltryptophan	200	55	52
Hypaphorine	50	76	72
Hypaphorine	200	25	26
4-Hydroxytryptophan	50	76	83
5-Hydroxytryptophan	50	100	100
α-Hydrazinotryptophan	50	58	47
α-Hydrazino-5-hydroxytryptophan	50	48	48
α-Methyl-5-hydroxytryptophan	150	110	134

* Adrenalectomized rats weighing 100–130 g were injected i.p. with the test compound, and tryptophan-3′-^{14}C was given by the same route on the contralateral side. In any given experiment, the animals were weight-matched to within 10 g. The results are expressed as the ratio of the percentage of administered radioactive carbon recovered as $^{14}CO_2$ from the expiratory gases of the experimental animal to the corresponding percentage for the control, the ratio being multiplied by 100; this represents the "Per cent of control" shown in the last two columns of the table.

The accelerated breakdown of tryptophan-3′-^{14}C after the injection of α-methyl-5-hydroxytryptophan in a dose of 150 mg/kg (0·67 m-mole/kg) was small by comparison with the effects of L-tryptophan,[22] DL-α-methyltryptophan[21, 22] or D-tryptophan (Table 1). α-Methyl-5-hydroxytryptophan had approximately the same stimulatory effect when tryptophan-2-^{14}C was substituted for the 3′-labeled compound. At lower doses of the analog (50 or 100 mg/kg) its stimulatory action was not observed.

Among the compounds that inhibited the oxidation of tryptophan-3′-^{14}C, α-hydrazinotryptophan and α-hydrazino-5-hydroxytryptophan had appreciable effects when injected at 50 mg/kg. Hypaphorine, the betaine of tryptophan, and 4-hydroxytryptophan were somewhat less effective at this dose. It is interesting that 5-hydroxytryptophan did not influence the oxidation at all and that 5-methyltryptophan inhibited the oxidation when given in a dose of 200 mg/kg and then only after the initial 2-hr period. Tryptophol was tested at a dose level of 50 mg/kg on several occasions but gave inconsistent results.

The effect of α-hydrazinotryptophan was tested in an experiment with other forms of labeled tryptophan, viz. the pyrrole and benzene ring-labeled amino acids. It is evident from the results in Fig. 1 that the α-hydrazino compound inhibited the oxidadation of two of the tryptophan radio-isomers, but had no effect on the benzene

ring-labeled isomer. The inhibitor affected the conversion of tryptophan-3′-^{14}C to $^{14}CO_2$ to a greater extent than the conversion of tryptophan-2-^{14}C. At 6 hr inhibition by the compound was 65 per cent in the case of the former radio-isomer, and only 44 per cent for the latter. These results may indicate that α-hydrazinotryptophan exerts an effect on the metabolism of tryptophan, perhaps at the level of tryptophan

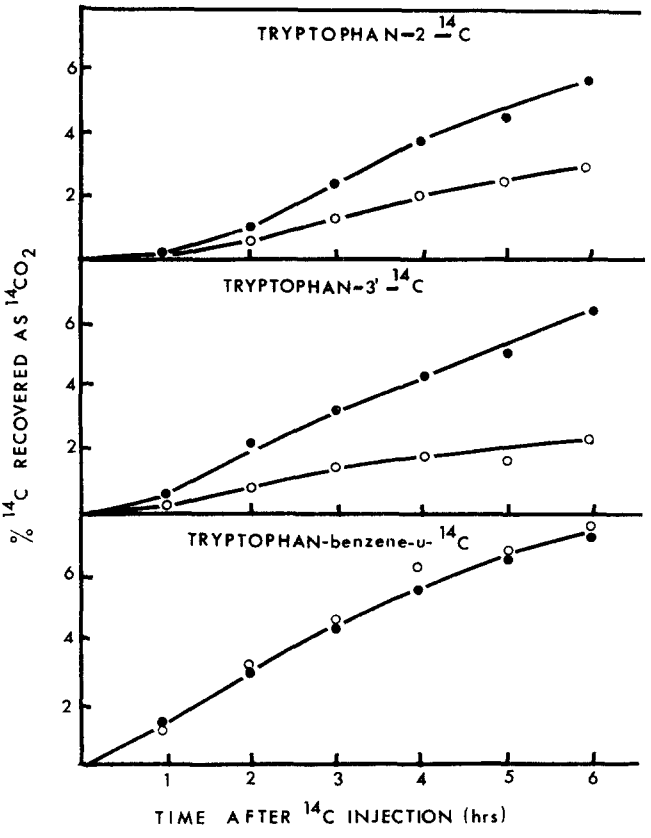

FIG. 1. The effect of α-hydrazinotryptophan on the rate of oxidation of DL-tryptophan labeled with ^{14}C in different positions. α-Hydrazinotryptophan (50 mg/kg) was injected i.p. into adrenalectomized rats and DL-tryptophan-^{14}C was then given by the same route on the contralateral side (○). Controls received 0·9% NaCl instead of α-hydrazinotryptophan (●). Each curve represents the mean of results with 4 different rats.

pyrrolase, formylkynurenine formamidase, or other enzymes located further along this pathway. Alternately the observations may be explained on the basis of inhibition of the enzymes concerned with the oxidation of formate derived from the pyrrole ring, or of alanine derived from the side chain. Formate-^{14}C and alanine-^{14}C were therefore injected into rats that had received α-hydrazinotryptophan at the same time. The oxidation of formate to respiratory $^{14}CO_2$ was reduced by only 12 and 18 per cent respectively in two experiments. The oxidation of alanine was reduced by 15 per cent.

α-Hydrazino-5-hydroxytryptophan was also tested further and was found to inhibit the oxidation of both tryptophan-2- and -3′-^{14}C. These inhibitions amounted to 37

and 52 per cent respectively at 6 hr, once again suggesting that the hydrazino derivative has more than one site of action, with the greater effect on the 3′-labeled amino acid.

These results, incidentally, demonstrate that the compounds tested cannot be acting simply on the tryptophan transport system, for then the oxidation of all three radioisomers would be reduced. Nevertheless, this point was assessed directly in the following manner. Rats were injected concurrently with α-hydrazinotryptophan (50 mg/kg) and tryptophan-3′-^{14}C i.p. Blood was removed from the tail vein at 15 and 60 min, and analyzed for radioactivity. The results are shown in Table 2. At 15 min both animals had similar amounts of radioactivity in the serum, but at 1 hr the control value was low, that for the drug-tested rats remaining high.

TABLE 2. EFFECT OF α-HYDRAZINOTRYPTOPHAN ON THE RADIOACTIVITY OF SERUM AFTER ADMINISTRATION OF TRYPTOPHAN-3′-^{14}C TO ADRENALECTOMIZED RATS*

Time after administration of labeled tryptophan (min)	Radioactivity of serum (cpm/ml)	
	Control	α-hydrazinotryptophan-treated
15	20,240†	22,940
60	8120	25,480

* For experimental details, see text.

† Each value represents the average of 2 animals.

Effect of tryptamine derivatives in vivo. Tryptamine and serotonin, given in low dosages to avoid serious pharmacological actions, had little or no effect on the oxidation of labeled tryptophan, as shown in Table 3. Of the two α-alkyltryptamines tested, α-ethyltryptamine demonstrated some inhibitory action on the catabolism of the methylene carbon of tryptophan to carbon dioxide. In the case of the two *N,N*-dialkyl-substituted tryptamines, the dimethyl compound was the more effective. The strongest inhibition was shown by *N*-acetyltryptamine.

TABLE 3. EFFECT OF TRYPTAMINE DERIVATIVES ON THE RATE OF OXIDATION OF TRYPTOPHAN-3′-^{14}C TO $^{14}CO_2$ IN ADRENALECTOMIZED RATS*

Compound	Dose (mg/kg)	Per cent of control at	
		2 hr	6 hr
Tryptamine	50	113	89
5-Hydroxytryptamine†	25	110	110
α-Methyltryptamine	50	93	85
α-Ethyltryptamine	50	63	80
N,N-dimethyltryptamine	50	46	52
N,N-diethyltryptamine	50	61	89
N-acetyltryptamine	50	26	41

* Conditions as described in Table 1.

† This animal received tryptophan-2-^{14}C.

Effect of analogs of tryptophan and tryptamine on tryptophan pyrrolase activity in vitro. Of the tryptophan derivatives studied, α-hydrazinotryptophan and tryptophol were the most potent inhibitors of the enzyme *in vitro*, as shown by the data of Table 4. Hypaphorine was a weak inhibitor of tryptophan pyrrolase at a concentration of 0·001 M. All three ring-hydroxylated derivatives of tryptophan—α-methyl-5-hydroxytryptophan, α-hydrazino-5-hydroxytryptophan and 4-hydroxytryptophan—lowered

TABLE 4. EFFECT OF TRYPTOPHAN ANALOGS ON TRYPTOPHAN PYRROLASE ACTIVITY *in vitro**

Compound	Per cent inhibition		
	No preincubation		Preincubation
	(5×10^{-4} M)	(10^{-3} M)	(10^{-3} M)
α-Hydrazinotryptophan	51	75	81
α-Hydrazino-5-hydroxytryptophan	27	50	58
Hypaphorine	7	18	
4-Hydroxytryptophan		51	
α-Methyl-5-hydroxytryptophan	15	36	
Tryptophol	86	92	95
Tryptamine		45	77
α-Methyltryptamine		21	31
α-Ethyltryptamine		24	46
N,N-diethyltryptamine		24	24
N-acetyltryptamine		79	23
Indole-3-acetic acid		24	0
Hydrazine		27	65

* Tryptophan pyrrolase in the liver of adrenalectomized rats was assayed as follows: Liver homogenate (final concentration in the reaction mixture, 4%, w/v) was added to the reaction mixture containing: 0·2 M sodium phosphate buffer, pH 7·0; 7×10^{-6} M hematin; and $1{\cdot}3 \times 10^{-3}$ M L-tryptophan. The reaction was stopped by adding metaphosphoric acid to a final concentration of 15%. Concentration of inhibitor is shown in the table. The reaction was always started by the addition of the substrate. Preincubated mixtures were held at 37° for 30 min before tryptophan was added. Controls with and without preincubation were used, and the per cent inhibition shown in the table is based on the appropriate control value.

the pyrrolase activity *in vitro* to about the same extent, whereas *in vivo* α-hydrazino-5-hydroxytryptophan had shown a much greater effect than the other two in respect to tryptophan catabolism (see above).

Several of the tryptamine derivatives, which diminished the rate of tryptophan oxidation *in vivo*, acted similarly *in vitro*; *N*-acetyl-tryptamine was the most effective as an enzyme inhibitor. However, tryptamine, which had been inactive *in vivo*, inhibited the enzyme by almost 50 per cent *in vitro*.

The results in Tables 1, 3 and 4 show that most derivatives that decrease the rate of oxidation of tryptophan *in vivo* inhibit tryptophan pyrrolase activity *in vitro* to a greater or lesser extent. It should be noted that tryptophol, which in certain experiments *in vivo* had shown some weak inhibitory effects, was a potent inhibitor of tryptophan pyrrolase *in vitro*. Moreover, α-methyl-5-hydroxytryptophan at the higher dosage used stimulated the catabolism of tryptophan (Table 1), but at lower dosages had a weak inhibitory action. It is possible that some of these results stem simply from slow transport of these compounds from the peritoneal cavity to the liver.

It appears that the α-amino group of the side chain is not necessary for inhibitory action *in vitro*, as exemplified by the results with the alcohol, tryptophol and the carboxylic acid, indole-3-acetic acid (Table 4). It was of interest to examine further the binding of these tryptophan derivatives to the enzyme, and several were preincubated with the enzyme for 30 min in the absence of substrate in order to determine whether the time of formation of the enzyme–inhibitor complex is appreciable. It was found (Table 4) that α-hydrazinotryptophan, its 5-hydroxy derivative, tryptamine, α-methyltryptamine, and α-ethyltryptamine, each containing a primary amino group, caused greater inhibition after preincubation than otherwise. On the other hand, tryptophol and *N,N*-diethyltryptamine did not inhibit any more strongly after preincubation with the enzyme preparation. In the cases of the amide *N*-acetyltryptamine and of indole-3-acetic acid, there was even less inhibition after preincubation than before. These results indicate that the indolic compounds studied react with the enzyme in more than one way. It should be noted that those compounds whose degree of inhibition increased upon preincubation may have exerted other actions on the enzyme, such as protection against spontaneous inactivation; this would not be manifested under the conditions of the assay *in vitro* used in this work because the inhibitor was not removed from the reaction medium. Those compounds like tryptophol and *N,N*-diethyltryptamine, which inhibit to the same extent whether preincubated with the enzyme extract or not, presumably come to equilibrium rapidly in forming an enzyme–inhibitor complex or, alternatively, produce a rapid irreversible inactivation of the enzyme. The third group of inhibitors, those that were less effective after preincubation (*N*-acetyltryptamine and indole-3-acetic acid), may be destroyed during the preincubation period by some enzyme(s) in the supernatant fraction of the liver that was used for its tryptophan pyrrolase activity. On the other hand, it is conceivable that they protect the enzyme from inactivation in the same sense that α-methyl-tryptophan does this,[13, 17] or that they convert latent[25] tryptophan pyrrolase to the active form.

Kinetic studies of the mode of inhibition of tryptophan pyrrolase by α-hydrazinotryptophan and tryptophol by the graphic method of Lineweaver and Burk[26] showed that both compounds inhibit by noncompetitive means. Tryptophan pyrrolase has hematin or methemoglobin[25] as its coenzyme, and in normal liver there is a mixture of holo- and apoenzyme, i.e. the enzyme is not ordinarily saturated with its coenzyme. α-Hydrazinotryptophan was examined as an inhibitor of tryptophan pyrrolase in the absence and in the presence of added hematin. The analog inhibited the endogenous holoenzyme by 54 per cent, but it inhibited the hematin-supplemented preparation by 75 per cent. This may indicate that the hydrazino compound prevents the binding of hematin to apoenzyme by reacting with one or the other of these components.

Because the method of determining the activity of tryptophan pyrrolase activity by the rate of formation of kynurenine involves the successive actions of pyrrolase and kynurenine formamidase, it was important to exclude the possiblity that α-hydrazinotryptophan was simply inhibiting the second enzyme so that under any conditions kynurenine formation would be prevented. Ordinarily the formamidase is present in great excess in the supernatant fraction of liver, but if it were inhibited, formylkynurenine would be expected to accumulate. This compound absorbs maximally at 321 mμ, whereas the peak for kynurenine is at 365 mμ. Hence the ratio of absorbances at 365 and 321 mμ would decrease if a compound were simply inhibiting the formamidase.

Experimentally it was found that the ratio for a control reaction mixture after incubation (i.e. after enzymic action) was 2·5. Use of α-hydrazinotryptophan in the reaction mixture provided the usual inhibition and the ratio of absorbances remained at the same value. On the other hand, when formanilide, a known inhibitor of kynurenine formamidase was used, the ratio of absorbances fell to 2·0, signifying the relative accumulation of excess formylkynurenine.

Effect of tyrosine and dopa derivatives in vivo. Six compounds generically related to tyrosine or to dopa were tested. The results in Table 5 show that only α-methyl-α-hydrazino-3,4-dihydroxyphenylpropionic acid and α-methyl-*m*-tyrosine retard the

TABLE 5. EFFECT OF AROMATIC α-METHYLAMINO ACIDS ON THE RATE OF OXIDATION OF TRYPTOPHAN-3′-^{14}C TO $^{14}CO_2$ IN ADRENALECTOMIZED RATS*

Compound	Dose (mg/kg)	Per cent of control at 2 hr	Per cent of control at 6 hr
α-Methyltyrosine	200	88	96
α-Methyl-*m*-tyrosine	180	85	80
α-Methyl-*m*-methoxyphenylalanine	200	132	143
	200†	87	87
L-α-Methyldopa	200	80	98
	200	91	98
α-Methyl-3,4-dimethoxyphenylalanine	200	126	121
α-Methyl-α-hydrazino-3,4-dihydroxyphenylpropionic acid	200	28	42
	100†	80	100
	200†	50	64

* Experimental conditions as in Table 1.

† Tryptophan-2-^{14}C was used in this experiment.

degradation of tryptophan. These compounds also inhibit tryptophan hydroxylase.[12] In these tests α-methyldopa was without effect on carbon dioxide formation from tryptophan in spite of the fact that it inhibits three enzymes that can affect the metabolism of tryptophan: dopa decarboxylase,[27] which also acts on 5-hydroxytryptophan; D-amino acid oxidase;[20] and tryptophan hydroxylase.[12, 28]

It is interesting that the hydrazino analog of α-methyldopa (Table 5) inhibits the oxidation of tryptophan-3′-^{14}C to a greater extent than the oxidation of the pyrrole-labeled compound. This result is in agreement with the effects of the two other hydrazino compounds studied, α-hydrazinotryptophan and α-hydrazino-5-hydroxytryptophan.

DISCUSSION

This study of the effects of amino acid analogs on the oxidation of tryptophan *in vivo* shows that many compounds can reduce the formation of respiratory $^{14}CO_2$ from tryptophan-^{14}C in adrenalectomized rats. Among the compounds possessing this property are secondary and tertiary amines including α-hydrazinotryptophan, α-hydrazino-5-hydroxytryptophan, *N*,*N*-dimethyltryptamine, *N*,*N*-diethyltryptamine and the amide *N*-acetyl-tryptamine. These were the most potent inhibitors of the

catabolism of tracer doses of labeled tryptophan *in vivo* to respiratory $^{14}CO_2$. In the case of certain inhibitors an attempt was made to establish at what phase in the formation of the radioactive carbon dioxide they act. The possibility that α-hydrazinotryptophan interferes with the transport of tryptophan from the peritoneal cavity through the blood to the liver was minimized by two different types of experiment. In the first place, the amount of radioactivity in the serum 15 min after the concurrent administration of labeled tryptophan and α-hydrazinotryptophan corresponds to the value found in the controls. Forty-five min later, the radioactivity in the serum remained at high levels in the drug-treated rats, but fell to low values in the controls (Table 2). This result implies either that tryptophan metabolism is inhibited by the hydrazino compound, resulting in an accumulation in the serum of radioactive label derived from tryptophan (if not an accumulation of the amino acid itself), or that the transport of tryptophan into some organs is diminished. The measurement of the amount of labeled tryptophan in liver and other organs would be necessary to establish this point.

The second experiment dealing with this matter compared the effects of the hydrazino derivative on the oxidation of three radio-isomers of tryptophan. The test compound retarded the formation of respiratory $^{14}CO_2$ from tryptophan-2- or -3′-^{14}C, but not from tryptophan that was uniformly labeled in the benzene ring (Fig. 1). If the transport of tryptophan were impeded by the presence of α-hydrazinotryptophan, one would expect all three radio-isomers to suffer the same decrease in rate of oxidation. Now, according to the accepted major pathway for the catabolism of tryptophan along the kynurenine pathway, the removal of ^{14}C from pyrrole-labeled tryptophan precedes the removal of the radioactive carbon atom from tryptophan labeled in the methylene carbon or in the benzene ring. Therefore, the extent of inhibition of oxidation with tryptophan-2-^{14}C as substrate would be expected to establish the level for the other isomers of tryptophan-^{14}C. Yet α-hydrazinotryptophan reduced the rates of oxidation of tryptophan-3′-, -2-, and -benzene ring-^{14}C in decreasing order of magnitude (Fig. 1). Because tryptophan labeled in the 3′-carbon was inhibited to a greater extent than tryptophan labeled in the pyrrole ring, it can be considered that α-hydrazinotryptophan has alternate sites of action. These sites would probably not involve a differential effect on the oxidation of formate or alanine because both of these compounds, studied independently of their origin in the tryptophan molecule, were inhibited only slightly and to the same extent.

Hydrazine derivatives can inhibit pyridoxine-dependent enzymes by reacting with the carboxyl group of pyridoxal phosphate. This may explain, at least in part, the inhibitory action of the hydrazino analogs of indolic amino acids on dopa decarboxylase,[29] as it does the similar action of the hydrazino analog of dopa and related compounds (cf. ref. 30). Furthermore, the removal of the label from tryptophan-3′-^{14}Cis accomplished through the action of the pyridoxal phosphate-linked enzyme kynureninase. Therefore, another possible site of action of α-hydrazinotryptophan is at the level of action of this enzyme.

α-Hydrazinotryptophan and α-methyl-α-hydrazino-β-(3,4-dihydroxyphenyl) propionic acid exhibited the same differential effect on the two forms of radioactive tryptophan (Fig. 1 and Table 5 respectively). It was found that α-hydrazino-5-hydroxytryptophan also inhibited the oxidation of tryptophan-3′-^{14}C to a greater extent than that of the pyrrole-labeled amino acid.

The lack of inhibitory effect of α-hydrazinotryptophan on the metabolism of tryptophan labeled in the benzene ring (Fig. 1) is difficult to explain on the basis of the accepted order of stepwise oxidation of tryptophan-carbons. Although the oxidation of the "formate" and "alanine" carbons of tryptophan is reduced by α-hydrazinotryptophan, this inhibition *in vivo* is not appreciably reflected in the metabolism of the benzene ring-labeled compound. If the inhibition were taking place after the separation of the labeled carbons from the respective aromatic molecules, there would be sufficient explanation for the lack of effect with this radio-isomer. Inhibition of exogenous formate and alanine was not as great as when these were provided as precursor tryptophan. Of course, when precursor is given, formate and alanine would appear intracellularly and the degree of inhibition with α-hydrazinotryptophan present in the liver cell might be greater than when the formate and alanine are provided by i.p. injection. Alternatively, a knowledge of the relative activities of the enzymes of the pyrrolase pathway *in vivo* may provide an explanation. Thus, a rate-limiting enzyme in the pyrrolase pathway would permit a small and constant quantity of its substrate to be degraded at the maximum velocity of the reaction. Even if the substrate concentration for this rate-limiting step were reduced by inhibition of earlier steps, the reaction might still operate at maximal velocity. Thus, inhibition at the outset of the pathway (pyrrolase, kynureninase) would not necessarily result in a reduction of the velocity of a rate-limiting reaction by reason of lack of substrate, and the apparent rate of oxidation of benzene ring-labeled tryptophan would remain unchanged. In support of this view, Ikeda *et al.* have shown[31] that the sp. act. of picolinic carboxylase is one-third as great as that of kynureninase in rat liver. This explanation is valid only if the relative concentrations of ^{12}C and ^{14}C intermediates remain unchanged in the experimental (i.e. drug-treated) animal. To clarify this, it would be desirable to measure specific labeled metabolites of the tryptophan radio-isomers in the liver at intervals after injection of labeled tryptophan and inhibitor. This form of study may prove to be a useful tool in localizing rate-limiting steps of simple pathways *in vivo*.

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