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METABOLISM OF α-METHYLTRYPTOPHAN*

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Abstract—The metabolism of α-methyltryptophan was studied in the rat. The α-methylamino acid was measured by colorimetric and radioactive methods (the latter used for tritium-labeled material) at various time intervals after its intraperitoneal injection. The specific uptake (μg/g organ) was highest in the kidneys, pancreas, and intestinal tract. The amino acid was detectable in these organs, as well as in the liver and plasma, for at least four days. There were low concentrations in the brain, spleen, heart, and lungs. α-Methyltryptophan caused an increased excretion of kynurenic and xanthurenic acids for two days after its administration, thus displaying an effect on the metabolism of endogenous tryptophan.

α-METHYLTRYPTOPHAN [DL-α-amino-α-methyl-β-(indolyl-3′-)propionic acid], when administered to rats, brings about an increased activity of the hepatic enzyme tryptophan pyrrolase.[1] It also causes a transient weight loss, thought to be the result of increased destruction of endogenous tryptophan.[2] α-Methyltryptophan and tryptophan cause this increased pyrrolase activity in the adrenalectomized,[3] as well as in the intact rat which distinguishes these two compounds from the numerous other substances that can do the same but require the presence of the adrenal cortex to effect the change in enzymic activity. However, there are some important differences in the effects of the two amino acids. Although tryptophan is a substrate for the enzyme, α-methyltryptophan does not appear to be so.[1] Another difference in the action of these two compounds lies in the length of time during which enzyme activity remains elevated above control levels: after the administration of tryptophan the activity of the enzyme increases to a maximum but returns to normal levels in less than 24 hr, whereas the effect of α-methyltryptophan persists for as long as one week.[4] In order to elucidate the action of α-methyltryptophan, its length of residence in the body and its effects on the metabolism of endogenous tryptophan, as measured by the excretion of kynurenic and xanthurenic acids, were studied.

METHODS AND MATERIALS

L-Tryptophan was purchased from California Corp. for Biochemical Research, Los Angeles, Calif. DL-α-Methyltryptophan·H_2O was donated by Merck, Sharp and Dohme Research Laboratories, Rahway, N.J. One gram of this substance was exposed to tritium gas in order to label it by the Wilzbach technique. The tritiated material was recrystallized several times, finally yielding 0·325 g. We are very much

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indebted to Dr. S. G. Mason and Dr. I. Wadhera of the Pulp and Paper Research Institute of Canada (Montreal) for carrying out the tritiation. The labeled material was chromatographed on Whatman 3MM chromatography paper (46 × 57 cm) that had been previously washed with water. The following solvents were used in succession: butan-1-ol:acetic acid:water (4:1:5); propan-2-ol:ammonia:water (20:1:2); water; 20% KCl (aqueous); butan-1-ol:acetic acid: water (12:3:5). After chromatography on the first solvent, two strips from either side of the paper were cut and scanned in an automatic windowless scanner. The radioactivity, corresponding in R_f to authentic α-methyltryptophan (as determined by ninhydrin stain) was eluted, redissolved in water, and applied to the paper in preparation for chromatography in the second solvent. The chromatographic separation and elution were repeated with successive solvents. The yield of tritiated material after this procedure (50 mg) was approximately 15% of the starting weight. The final material used in these experiments showed a peak with a slight bifurcation in the fifth solvent run, at R_f 0·63. The R_f of unlabeled α-methyltryptophan in the same run was 0·60. The specific activity of the labeled amino acid was 2·04 mc/mmole.

Male rats of the Sprague-Dawley strain were used; specific weights are presented in the description of given experiments. After administration of the appropriate compound, the animals were killed at intervals. Blood plasma was collected and the liver, brain, pancreas, kidney, and other organs, as specified, were rapidly removed, weighed, and homogenized in 5 to 10 volumes of ice-cold distilled water. After precipitation of proteins with a saturated solution of picric acid, and centrifugation, the supernatant solutions were examined colorimetrically for indoles by the method of Dickman and Crockett.[5] In this method tryptophan and α-methyltryptophan react with xanthydrol to produce a colour that absorbs maximally at 500 mμ. Other indoles, including those derived from the administered compounds, are also chromogenic. Hence the results in animals receiving the amino acids were always compared with untreated controls. It has been assumed that the excess of indoles above control levels represents the administered amino acid, together with its indolic metabolites. In the case of α-methyltryptophan, α-methyltryptamine is known to be formed from it by decarboxylation.[6]

In an alternative procedure, blood was taken from rats by cardiac puncture and was oxalated. A sample of 0·5 ml of the oxalated plasma was made up to 2·5 ml with water; then 0·5 ml of 6 N H_2SO_4 and 0·5 ml of 10% sodium tungstate were added. The mixture was centrifuged to separate the precipitated proteins. The protein-free supernatant was passed through a column of Dowex 50W-X8 (2·5 cm high, 1·0 cm in diameter) that had been washed with a solution containing triethylamine:methanol: water (3:2:3), followed by 5 N HCl and water, and finally equilibrated with 0·2 M phosphate buffer, pH 7·4. The retained substances were eluted with a mixture of triethylamine:methanol:water, (3:2:3). The solvent was then evaporated by warming under a stream of air. The residue was taken up in a minimum volume of ethanol and applied to a thin layer of cellulose powder for chromatography in butan-1-ol: acetic acid: water (6:1:3) in the first dimension, followed by 20% KCl in the second. The amino acid was detected after spraying the plates with van Urk's reagent—1% *p*-dimethylaminobenzaldehyde in 25% HCl:ethanol 96% (1:1). By chromatographing known amounts of the amino acid under the same conditions it was possible to prepare a standard curve.

Kynurenic and xanthurenic acids were measured in the urine of rats by colorimetric[7] and spectrophotofluorometric[8] methods.

When α-methyltryptophan-^{3}H was administered, the removed organs were examined by the method of Herberg.[9] Liver, pancreas, brain, kidneys, intestinal tract and, in some animals, spleen, lungs, and heart were homogenized in 50 volumes of ice-cold water, and the homogenates were lyophilized. Ten mg of the dried powder was dissolved in 1 ml of methanolic hydroxide of Hyamine (Packard Instrument Co., Des Plaines, Ill.) and then incubated in a Dubnoff metabolic shaker at 55° for 30–60 min; the longer times were needed for the less soluble powders. Fifteen ml of phosphor solution [2,5-diphenyloxazole, 0·5%, and 1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene, 0·01%, in toluene] were added, and the solution was counted in a liquid scintillation spectrometer (Packard Instrument Co.). Quenching was determined by adding an internal standard of tritiated toluene.

RESULTS

Plasma indoles after administration of α-methyltryptophan

Individual rats (100-150 g) were killed at various times after the injection of α-methyltryptophan (100 mg/kg) and samples of blood were taken. The α-methyltryptophan was separated on a cation-exchange resin followed by thin-layer chromatography on cellulose, as described above, and its concentration was determined. The results are shown in Fig. 1 where it is seen that the amino acid falls from the high values observed shortly after the injection to about 0·4 mg/100 ml at the fourth

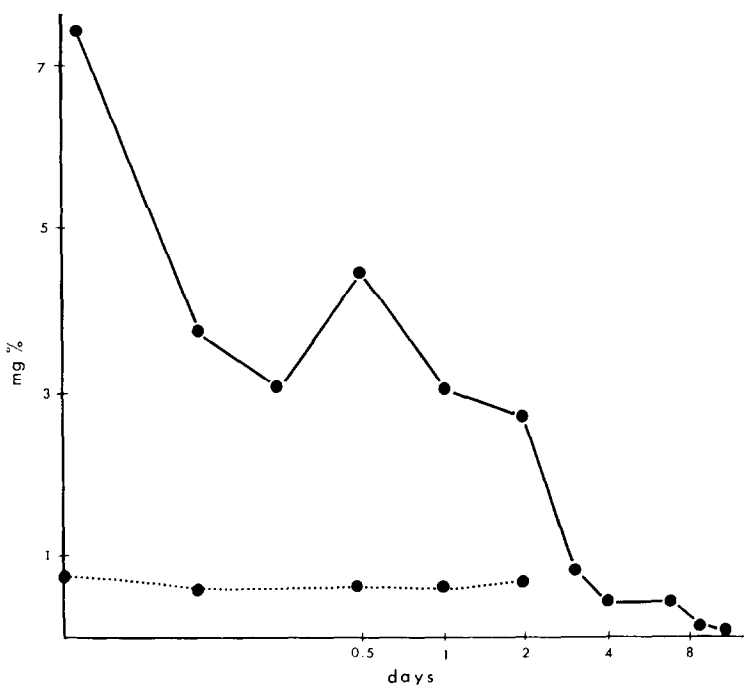

FIG. 1. Concentration of tryptophan and α-methyltryptophan in plasma. Male rats were injected with α-methyltryptophan (1 mmole/kg) i.p., and were killed at the times indicated. α-Methyltryptophan ●——●, tryptophan ●·····●. The abscissa is logarithmic.

day. Some was still detectable one week after injection, but not at later times. The concentration of tryptophan in plasma was measured at the same time; it is evident from the curve in Fig. 1 that the administration of α-methyltryptophan did not influence the level of the endogenous amino acid between the hours 1 and 48 after injection (Cf. induction by cortisol[10]).

Indoles in various organs after administration of L-*tryptophan and* DL-α-*methyltryptophan*

The concentration of indoles in liver, brain, pancreas, and kidneys was measured by the xanthydrol reaction, after homogenization of the tissues and removal of protein. After the i.p. administration of 1 mmole tryptophan/kg to rats weighing 120–130 g, the concentration of the amino acid in the liver was above control levels for several hours, but fell to normal values in 12 hr (Fig. 2). On the other hand, the concentration of α-methyltryptophan (and/or its indolic metabolites) remained elevated for more than four days.

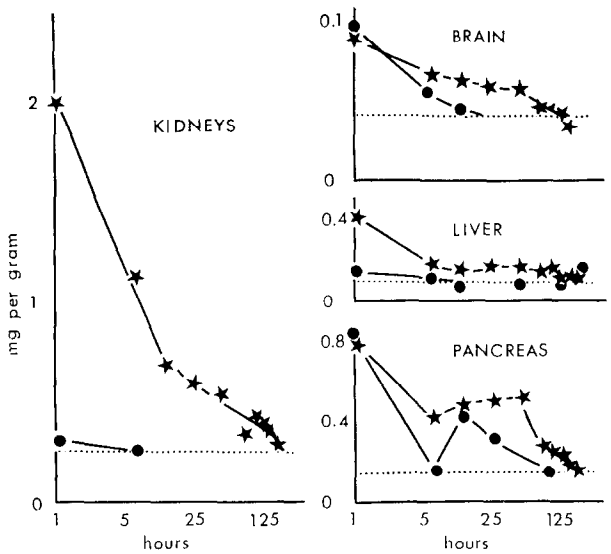

FIG. 2. Concentration of tryptophan and a-methyltryptophan in organs of rats. Male rats were injected i.p. with 1 mmole tryptophan/kg, ●——●; 1 mmole α-methyltryptophan/kg, ★——★; controls (....) received 0·9% NaCl. The ordinate denotes the concentration of the amino acids, as mg/g wet weight of tissue. The abscissa indicates the time after injection, on a logarithmic scale.

The pancreas showed high concentrations of both amino acids within the first hour after injection; the concentrations fell to a low point at 6 hr and then rose once more, providing new peaks (Fig. 2). In this connection the pancreas is recognized as a site of rapid protein synthesis, and a rapid uptake of amino acids can be expected in that organ. This phenomenon resembles the accumulation by the pancreas of another synthetic amino acid, 1-aminocyclopentanecarboxylic acid, as reported by Berlinguet and his colleagues.[11] This amino acid, like α-methyltryptophan, lacks an α-hydrogen, a fact that limits the number of possible routes of metabolism. Although 1-aminocyclopentanecarboxylic acid is rapidly taken up, it is not incorporated into pancreatic protein. The secondary rise in the concentration of amino acids may represent accumulation of the compounds being released from other organs where

they had been absorbed earlier. It may also be related to the realimentation begun after the sixth hour, the animals having been deprived of food for 20 hr previously.

The entry of xanthydrol-positive material into the brain was not as marked as in the case of liver and pancreas, but there nevertheless were increases following both tryptophan and α-methyltryptophan. The increase occasioned by injection of the latter compound persisted for many days (Fig. 2).

The kidneys contained very large amounts of indoles derived from the injection of α-methyltryptophan. Again, this excess persisted for almost one week, the decrease during that time occurring at a more or less logarithmic rate (Fig. 2). In the case of tryptophan, indolic substances attained normal levels within 5 hr.

Distribution of ^3H-α-methyltryptophan

Because of the unusually long residence of α-methyltryptophan and its metabolites in the body, as observed in the above experiments, it was decided to investigate this further by employing the more sensitive methods available with a tritiated product. As the amount of ^3H-α-methyltryptophan was limited, 8 μc was injected into each of three rats, weighing 60–62 g each. The animals were killed 6, 24, and 120 hr.

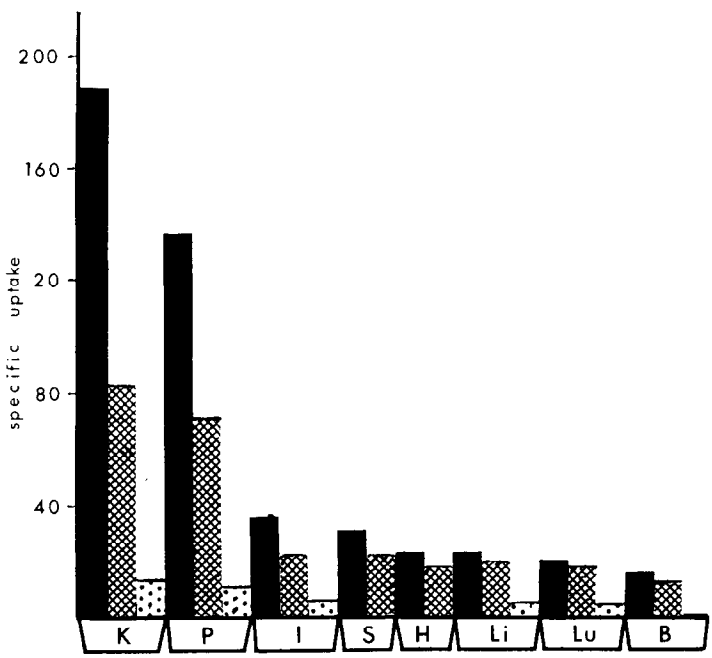

FIG. 3. The measurement of ^3H-α-methyltryptophan in organs. Three male rats were injected with 10^6 cpm of ^3H-α-methyltryptophan and killed at 6 hr (solid bar), 24 hr (cross-hatched bar), 5 days (dotted bar). The ordinate indicates specific activity of each organ expressed as cpm/mg dried weight of tissue. The organs are: K, kidney; P, pancreas; I, intestine; S, spleen; H, heart; Li, liver; Lu, lung; B, brain.

respectively, after this injection, and the liver, pancreas, brain, kidneys, lungs, intestinal tract, spleen, and heart were removed and treated as described under Methods and Materials. The concentrations of labeled material (cpm/mg dry matter) are shown in Fig. 3. The highest values were found in the kidneys and pancreas, followed by

the intestine. Spleen and heart had high values initially, but no radioactivity could be detected in these organs at 120 hr. The lungs, brain and liver had low concentrations of tritiated material, although the absolute amount in the liver was relatively great because of the mass of that organ. It is interesting that the radioactivity in these three organs was lost slowly, at least up to 24 hr (Fig. 3).

The results of these experiments were compared with the data obtained when unlabeled material (in larger doses) had been injected. The comparison is set out in Table 1 where the concentration of the xanthydrol-positive material and the radioactivity, respectively, in pancreas, brain, and kidneys are expressed as ratios of the values found for liver. The closest agreement in results was observed at 24 hours. The greater discrepancies at 6 hr may be accounted for by the rapid changes in concentration taking place at this period (cf. Fig. 2) and the fact that the value for labeled material is based on but one animal per time period. Even after five days, when the values are now very low, the ratios in the two columns are of the same order.

The acid-insoluble fraction of the liver, pancreas, and intestinal tract was also examined by counting, but very low values were obtained, of the order of 10 to 40 counts above background. It has not been determined whether these counts represent residual contamination or ^{3}H-exchange, or whether they stem from a small but definite incorporation of α-methyltryptophan into protein.

TABLE 1. CONCENTRATION OF α-METHYLTRYPTOPHAN IN RAT ORGANS

Time after injection	Organ	Experiments with α-methyltryptophan*	
		Tritiated	Unlabeled
6 hr	Pancreas	6·8	2·4
	Kidney	9·0	5·6
	Brain	0·7	0·7
24 hr			
	Pancreas	3·0	1·7
	Kidney	4·0	3·4
	Brain	0·6	0·6
5 days			
	Pancreas	6·1	1·0
	Kidney	6·0	3·3
	Brain	0·3	0·2

* Three rats (60–62 g) were injected with ^{3}H-α-methyltryptophan as described in the text. The radioactivity found at the stated times in their organs is given as a ratio (liver, 1·0). Twelve rats were injected with 1 mmole of unlabeled α-methyltryptophan per kg, and indolic material was determined on the extracted organs by means of a xanthydrol reagent (see text). Figures are ratios, relative to the value found for liver. Each figure is the average for 4 animals.

Excretion of quinaldic acids

Kynurenic and xanthurenic acids were measured in the urines of rats (120–150 g) receiving an i.p. injection of α-methyltryptophan (1 mmole/kg). The urines were collected for 24 hr beginning at various times after the injection, except in the case of the 12-hr experiment. Results shown in Fig. 4 indicate that rats receiving the α-methyl amino acid excrete a larger amount of kynurenic acid than do controls, and this effect is measurable for at least two days. The same result was obtained for xanthurenic acid (Fig. 4).

At five days both quinaldic acids dropped below control values in the urine. This may be brought about by a mild tryptophan deficiency developed during the course of the five days when pyrrolase levels were high.[2, 4]

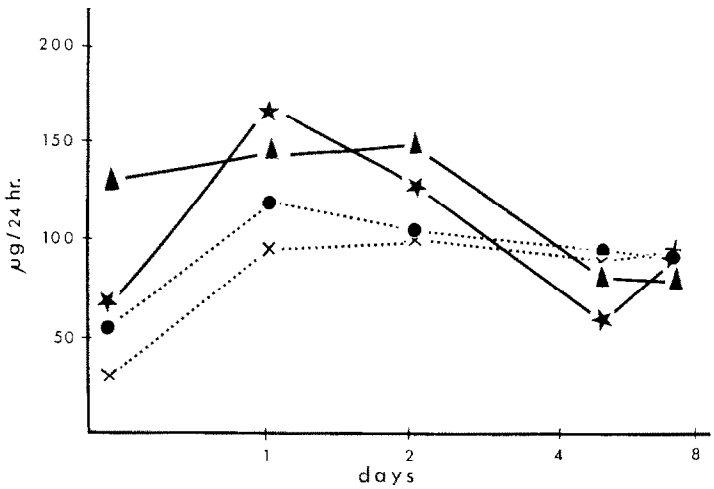

FIG. 4. The excretion of kynurenic and xanthurenic acids in urine. The ordinate indicates rate of excretion, as μg/24 hr. The first set of points denotes values for 12-hr collection. The abscissa is scaled logarithmically as number of days after injection. Male rats were injected with α-methyltryptophan (1 mmole/kg): kynurenic acid ▲——▲, and xanthurenic acid ★——★ were measured. Controls received 0·9% NaCl: kynurenic acid ●·····●, xanthurenic acid ×·····×.

DISCUSSION

Sankoff and Sourkes[2] showed that rats injected with α-methyltryptophan maintain high levels of tryptophan pyrrolase, even up to seven days after injection. It is evident from our results that the compound, or its indolic derivatives can be detected in the plasma and liver for many days after injection. Hence the tryptophan pyrrolase activity may well be elevated because of the presence in the cell of the "inducing" amino acid for this period. The α-methyltryptophan could sustain a high level of pyrrolase by any combination of known mechanisms: stimulation of synthesis of apoenzyme *de novo*,[12] facilitation of combining capacity of the apoenzyme with its cofactor, hematin,[13, 14] or stabilization of the active enzyme by preventing its degradation.[1, 15, 16]

It appears from this work that α-methyltryptophan is concentrated differently by various organs of the rat, and most of it passes into the acid-soluble, non protein compartment of the cell. One cannot decide on the basis of the present data whether α-methyltryptophan is incorporated into protein, for less than 3% of the radioactivity of liver, pancreas, and intestine after administration of ^{3}H-α-methyltryptophan was found in the trichloroacetic acid-precipitable substances.

It has been suggested that the observed weight loss following an injection of the compound stems from a relative deficiency of the essential amino acid tryptophan, through rapid degradation of the endogenous supplies catalyzed by the induced enzyme. Moran and Sourkes[4] showed that exogenous tryptophan is metabolized at a faster rate when animals are injected with α-methyltryptophan. Presumably this is

due to the effects of the compound on tryptophan pyrrolase. In the present work kynurenic and xanthurenic acids were measured in an effort to determine whether the metabolism of endogenous tryptophan is also affected by α-methyltryptophan, for an increased output of these compounds is taken to mean either a metabolic block at the stage of kynureninase action[17] or a relatively high rate of catabolism of tryptophan to the stage of kynurenine and 3-hydroxykynurenine with funneling of some of the excess of these amino acids into the quinaldic acids. The observed increase in output of kynurenic acid and xanthurenic acid may be explained either way, although the former mechanism has been attributed to deficiency of pyridoxine,[18–20] a condition that does not apply to animals used in this work. It seems reasonable to conclude that α-methyltryptophan increases the rate of catabolism not only of exogenous tryptophan[4] but of the endogenous amino acid as well.

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