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PRELIMINARY COMMUNICATIONS

Convulsions and elevation of tissue citric acid levels induced by 5-fluorotryptophan

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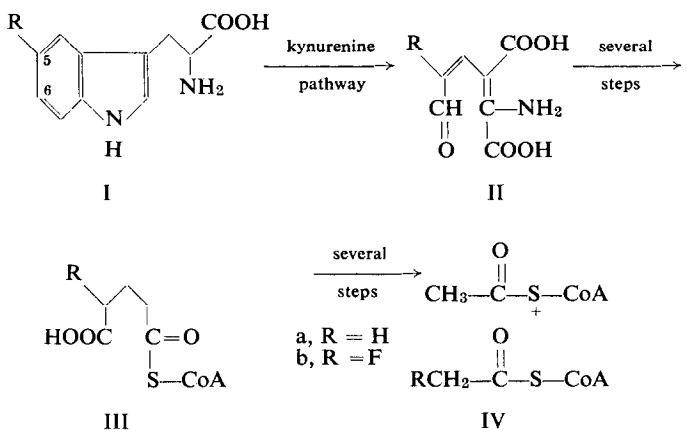
IT HAS recently been concluded[1] that the potent convulsant and lethal properties of *m*-fluorophenylalanine[2] and *m*-fluorotyrosine[2, 3] are probably mediated by the known toxic compound, fluoroacetic acid,[4–6] formed by the degradation of these amino acids via the normal tyrosine (transaminase) metabolic pathway.[7] A noteworthy implication of this conclusion is that other fluorinated aromatic amino acids might also lead to fluoroacetic acid as a result of the specialized metabolism characteristic of the parent (nonfluorinated) amino acids. Tryptophan is metabolized in the body mainly by the kynurenine pathway which, in part, yields products such as quinolinic acid and picolinic acid.[7] A substantial portion of tryptophan metabolism also includes complete degradation of the benzene moiety of the indole ring by the conversion of 2-acroleyl-3-aminofumaric acid (IIa) through several steps to glutaryl-coenzyme A (IIIa), each molecule of which then yields two molecules of acetyl-coenzyme A (IVa).[8] Metabolic studies with DL-tryptaphan-5-^{14}C have indicated that the C-5 atom of tryptophan is ultimately converted to C-2 of acetic acid.[9] This suggests that 5-fluorotryptophan (Ib) should yield fluoroacetyl-coenzyme A (IVb) and thereby induce convulsions and increases in tissue citric acid concentrations.



In toxicity studies, mice in groups of five were injected i.p. with DL-5-fluorotryptophan at doses ranging from 316 mg/kg to 1000 mg/kg; DL-6-fluorotryptophan at 1000 mg/kg; or DL-tryptophan at 1000 mg/kg. Beginning at about 4 hr after treatment with 5-fluorotryptophan at 1000 mg/kg i.p., the dramatic convulsant symptoms characteristic of *m*-fluorophenylalanine and *m*-fluorotyrosine were seen: tremors, swaying, slow respiration, opisthotonos, and repeated hind limb clonic and tonic extensor convulsions; death occurred at about 5 hr after treatment. At 562 mg/kg i.p., only two of five mice convulsed; no mice convulsed at 316 mg/kg. The groups treated with 1000 mg/kg of 6-fluorotryptophan or tryptophan were asymptomatic.

In citric acid studies, whole brains and kidneys were removed 4 hr after treatment and rapidly frozen in a dry ice–acetone bath. Citric acid levels were assayed within 24 hr by a modification of the procedure of Natelson *et al.*,[10] with pooled tissues from three mice per determination. 5-Fluorotryptophan caused a marked elevation of citric acid levels at 1000 mg/kg i.p. and a smaller but

significant increase at 316 mg/kg i.p. (Table 1). In contrast, the tryptophan- and 6-fluorotryptophan-treated mice showed no change in citric acid levels compared with control levels.

The present findings suggest that the pharmacological effects of 5-fluorotryptophan observed on acute treatment may be caused by the generation *in vivo* of fluoroacetyl-coenzyme A, which is subsequently metabolized to fluorocitrate. As shown by Peters and co-workers[6] the latter compound

TABLE 1. TISSUE CITRIC ACID LEVELS AND TOXICITY IN MICE

Compound	Citric acid concentration*			LD_{50}†
	Dose (mg/kg)	Brain (μg/g)	Kidney (μg/g)	(mg/kg)
Control (saline)		18 ± 1 (18)	14 ± 1 (15)	
DL-Tryptophan	1000	20 ± 4 (5)	9 ± 1 (5)	>1000
DL-5-Fluorotryptophan	316	47 ± 6 (5)	64 ± 15 (5)	776 (627–938)
	1000	159 ± 21 (10)	401 ± 83 (10)	
DL-6-Fluorotryptophan	1000	14 ± 1 (5)	18 ± 3 (5)	>1000

* Four hr after i.p. administration of the tryptophans (Nutritional Biochemicals Corp.). Concentrations are averages ± S.E. for the number of determinations given in parentheses.

† Based on deaths 24 hr after i.p. administration. Numbers in parentheses are 95 per cent confidence limits.

inhibits the aconitase step in the tricarboxylic acid cycle, thereby causing toxic effects and a concomitant accumulation of citric acid in tissues. The large dose of 5-fluorotryptophan needed to observe convulsions, death, and increases in citric acid levels is consistent with the complex multistep pathway required for the conversion of C-5 of tryptophan to C-2 of acetic acid. 6-Fluorotryptophan, which lacks the convulsant and citric acid-elevating properties of 5-fluorotryptophan, probably cannot yield fluoroacetic acid, possibly because its degradation by the kynurenine pathway is terminated at 4-fluoro-3-hydroxyanthranilic acid. It may be anticipated from the results presented that appropriately fluorinated intermediates in the tryptophan kynurenine pathway to acetic acid will also elicit convulsions and elevation of tissue citric acid levels. The selective blockade of these effects could enable detection *in vivo* of inhibitors of the different metabolic steps in the degradation of tryptophan.

Final confirmation of the toxic mechanism proposed above will require identification of fluoroacetate or fluorocitrate in the tissues of animals poisoned with 5-fluorotryptophan. Nevertheless, the present findings appear to support the previous contention[1] that fluorinated aromatic compounds can yield fluoroacetic acid and its resulting toxic effects if suitable metabolic pathways are available. Furthermore, they illustrate that toxicity dependent on fluoroacetic acid need not be restricted to compounds with low LD_{50} values, because lethal doses may depend on the complexity of metabolic pathways leading to fluoroacetyl-coenzyme A.

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REFERENCES

1. A. WEISSMAN and B. K. KOE, *J. Pharmac. exp. Ther.* In press.
2. P. D. BOYER, R. J. EVANS and P. H. PHILLIPS, *J. Pharmac. exp. Ther.* **73**, 176 (1941).
3. G. LITZKA, *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.* **183**, 427 (1936).
4. M. B. CHENOWETH, *Pharmac. Rev.* **1**, 383 (1949).
5. F. L. M. PATTISON, *Toxic Aliphatic Fluorine Compounds.* Elsevier, Amsterdam (1959).
6. R. A. PETERS, *Advanc. Enzymol.* **18**, 113 (1957).
7. A. MEISTER, *Biochemistry of the Amino Acids*, 2nd ed. Academic Press, New York (1965).

8. R. K. GHOLSON, Y. NISHIZUKA, A. ICHIYAMA, H. KAWAI, S. NAKAMURA and O. HAYAISHI, *J. biol Chem.* **237**, PC 2043 (1962).
9. G. P. MATHUR, C. Y. NG and L. M. HENDERSON, *J. biol. Chem.* **239**, 2184 (1964).
10. S. NATELSON, J. B. PINCUS and J. K. LUGOVOY, *J. biol. Chem.* **175**, 745 (1948).

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Relationship between the metabolic effects and the pregnancy-interrupting property of 6-azauridine in mice*

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IT HAS been observed previously that both 6-azauridine (AzUR)[1] and 6-azacytidine,[2] antagonists of pyrimidine synthesis *de novo*,[3,4] are capable of interrupting pregnancy in both mice and rats.[5–7] The antimetabolites have their optimal effect when administered soon after implantation of the fertilized ovum has occurred; a single dose of AzUR (500 mg/kg), without producing any toxic effects in the host, results in complete resorption of the embryos. During the second half of pregnancy, even repeated administration of AzUR does not produce consistently an interruption of pregnancy. In an attempt to understand the difference between the effects of AzUR upon the early and late stages of fetal development, we have compared the metabolic transformation and biochemical effects of AzUR in 6-day embryos and in 15-day fetuses of mice.

Animals. Pregnant albino mice were obtained from the Charles River Breeding Laboratories, Inc., North Wilmington, Mass.

Compounds. 6-^{14}C-Orotic acid hydrate (4·9 μc/μmole) and ^{14}C-carboxyl-orotic acid hydrate (35 μc/μmole) were obtained from New England Nuclear Corp., Boston, Mass. 4,5-^{14}C-6-Azauridine (3 μc/μmole) was obtained through the courtesy of Prof. F. Šorm and Dr. J. Škoda, Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague.

Effect of AzUR *upon orotic acid-incorporation* in vivo *in the fetus.* Mice, in days 6 and 15 of pregnancy, were injected i.p. with 6-^{14}C-orotic acid (4 μc and 8 μc respectively), together with AzUR (500 mg/kg); control mice received 6-^{14}C-orotic acid and saline. After 2 hr, the mice were sacrificed by decapitation and the uteri were excised. After removing the embryos from the uteri, the tissue was homogenized in cold 5% trichloroacetic acid (TCA). The homogenate was centrifuged and the precipitate, after being washed with TCA four times, was extracted with ethanol:ether (3:1) until the supernatant fraction was clear. The nucleic acids were extracted by heating the precipitate with 5% TCA at 95° for 30 min. After subsequent centrifugation, the TCA was extracted from the supernatant fluid by repeated shaking with ether. The optical density (260 mμ) and the radioactivity of the supernatant fraction were measured. The results are expressed as counts/min per ml/OD_{10}.

Phosphorylation of AzUR *in the embryos* in vivo. One hour after the i.p. administration of 5 μc of 4,5-^{14}C-6-azauridine, the mice were sacrificed and the embryos removed. The tissues were homogenized in 0·4 N perchloric acid and centrifuged, and the supernatant material was then neutralized with 8 N KOH. The resulting supernatant fraction was applied to Whatman 1 filter paper, and the phosphorylated AzUR was separated; a mixture of *n*-butanol, glacial acetic acid and water (10:1:3) was used.

Preparation of particle-free supernatant material from embryos. The embryos or fetuses, aged 6 and 15 days, respectively, were homogenized in cold 0·15 M KCl. The homogenate was centrifuged at 105,000 *g* for 90 min, and the resulting supernatant fraction was used as a source of enzyme activity for the reaction described in the following paragraph.

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