reported that rabbit aldehyde oxidase (aldehyde : oxygen oxidoreductase, EC 1.2.3.1) has the ability to oxidize allopurinol to oxipurinol *in vitro*. Furthermore, Huff *et al.*[7,8] showed that there is a sex difference of aldehyde oxidase activity in mice when *N*-methylnicotinamide was used as substrate. Therefore we are now investigating the relationship between allopurinol oxidizing enzyme and aldehyde oxidase in mice.

Elion *et al.*[2,9] reported that allopurinol is rapidly oxidized *in vivo* to oxipurinol which also inhibits xanthine oxidase. Previously we reported that the ^{14}C-compounds disappear much slower in male than in female mice after labeled allopurinol treatment. Thus it seems that this sex difference in allopurinol oxidizing activity may, to some extent, be concerned with acute toxicity.

Further work is needed to clarify this problem.

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Department of Pharmacology,
Faculty of Pharmaceutical Sciences,
Osaka University, Toneyama
Toyonaka,
Osaka, Japan

Heitaroh Iwata
Itaru Yamamoto
Keun Huh

Biochemical Pharmacology, Vol. 23, pp. 1146–1147. Pergamon Press, 1974. Printed in Great Britain.

Inability of rat brain homogenate to oxidize amphetamine

(*Received* 20 *July* 1973; *accepted* 17 *September* 1973)

In a recent series of papers in this journal Guha and Mitra[1–3] have reported that homogenates of rat and guinea pig brain catalyze the reduction of neo-tetrazolium chloride (NTC) in the presence of amphetamine. The system has been described as an amphetamine dehydrogenase; the most recent report suggests that tranylcypromine is also actively dehydrogenated by this system.[3] However, the activity of the enzyme system has been estimated by measuring the production of diformazan from NTC. The authors have not determined that a degradation product of amphetamine is produced in the course of the reaction.

Hucker[4] and Parli and McMahon[5] have recently reported on the metabolism of amphetamine with no evidence for a dehydrogenase pathway. Earlier work from this laboratory, using [^3H]labeled amphetamine, had shown that rats convert the drug *in vivo* by *p*-hydroxylation and oxidative deamination.[6] The present communication reports on attempts to measure the production of metabolites of [^3H]amphetamine by the rat brain dehydrogenase system as described by Guha and Mitra.[1–3]

Brains were removed from adult (300–400 g) male, Sprague–Dawley rats purchased from Murphy Breeding Laboratories, Plainfield, Ind., and homogenates prepared according to Mitra and Guha.[3] *d*-Amphetamine phosphate was supplied by the Pennwalt Corp., [^{3}H]*d*-amphetamine sulfate was purchased from the New England Nuclear Corp., NTC from the Sigma Chemical Co. and diformazan from Nutritional Biochemicals Corp.

Incubations were performed according to Mitra and Guha.[3] Diformazan production was measured by the method of Lagnado and Sourkes[7] using a Gilford model 2000 absorbance recorder with a Beckman model DU monochromotor. [^{3}H]amphetamine was assayed as described by Maickel *et al.*,[6] using a Packard Tri-Carb model 2425 liquid scintillation system. Metabolites of [^{3}H]amphetamine were separated by TLC on Silica gel G plates (0·25 mm, Brinkmann MN-Polygram) in the system described by Hucker.[4] Phenyl-2-propanone was visualized by spraying with 1% $KMnO_4$ in 5% aqueous Na_2CO_3. TLC plates were scanned for radioactive spots in a Packard model 7200 radiochromatogram scanner.

The conversion of NTC to diformazan in the system of Guha and Mitra[3] occurred with or without the addition of amphetamine. In an attempt to prepare a standard curve for the absorbance of diformazan at 520 nm, it was found that absorbance is not linear over the concentration range reported by Guha and Mitra.[1, 3] No amphetamine metabolites were observed when the incubate was extracted with methylene chloride and examined by TLC.[4] Incubation of [^{3}H]amphetamine with the homogenate and NTC, followed by extraction, TLC and radiochromatogram scanning indicated that greater than 95 per cent of the radioactivity present was contained in a spot corresponding to unchanged [^{3}H]amphetamine. In a definitive experiment, incubation mixtures were prepared with and without homogenate. Duplicate mixtures of each type were either incubated and extracted after 30 min or not incubated, i.e. extracted at $t = 0$. [^{3}H]amphetamine was extracted by a specific procedure and measured by liquid scintillation spectrometry.[6] Neither the presence of brain homogenate nor incubation at 37° for 30 min resulted in any reduction in the amount of [^{3}H]amphetamine that could be extracted. Based on the rate of diformazan production reported by Mitra and Guha,[3] one would have expected at least 85 per cent conversion of amphetamine. As little as 10 per cent metabolism of amphetamine would have been easily detected.

Many substances are known to reduce neo-tetrazolium chloride, ranging from simple alkaline pH to ascorbic acid, reducing sugars and flavoprotein enzymes.[8] Indeed, it was observed that during an early step in the amphetamine extraction procedure[6] at which the aqueous phase was made alkaline, the color characteristic of diformazan appeared in mixtures containing NTC and homogenate with or without amphetamine; mixtures containing only amphetamine and NTC developed no color. The reduction of NTC under these conditions appears to be a function of the presence of brain homogenate and not of amphetamine concentration.

In summary, an attempt was made to measure the production of [^{3}H]amphetamine metabolites by the rat brain amphetamine dehydrogenase system of Mitra and Guha.[3] No metabolic conversion of amphetamine was observed.

Acknowledgements—This work was supported by USPHS grants K02MH-41083 and MH-18852 and by NASA grant 15-003-117.

Department of Pharmacology,
Medical Sciences Program,
Indiana University,
Bloomington, Ind. 47401, *U.S.A.*

ROGER P. MAICKEL
STEADMAN D. HARRISON, JR.

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