diamine oxidase activity,[16] while thiamine is also a weak inhibitor of these enzymes, *in vitro*. It has been proposed that *in vivo* thiamine inhibits both of these enzymes.[15, 16] Thus, there is both precedent for the theory presented and a wider scope for a mechanism which would delay the catabolism of neurohumors such as acetylcholine, histamine, serotonin, and the catecholamines.

*Department of Pharmacology,*
*Yale University School of Medicine,*
*New Haven, Conn., U.S.A.*

H. Y. MELTZER

## REFERENCES

1. H. Y. MELTZER, Unpublished observations.
2. I. B. WILSON and S. GINZBURG, *Biochim. Biophys. Acta.* **27**, 196 (1958).
3. E. M. GAL and P. A. DREWES *Proc. Soc. Exp. Biol., N.Y.* **106**, 295 (1961).
4. J. R. DI PALMA and P. HITCHCOCK, *Anesthesiology* **19**, 762 (1958).
5. S. K. WOLFSON JR. and S. ELLIS, *Fed. Proc.* **13**, 418 (1954).
6. S. B. GERTNER, *J. Pharmacol.* **131**, 223 (1960).
7. T. CESARMAN, *Ann. N. Y. Acad. Sci.*, **80**, 988 (1959).
8. H. WEISSBACH, T. E. SMITH, J. W. DALY, B. WITKOP, and S. UDENFRIEND, *J. Biol. Chem.*, **235**, 1160 (1960).
9. H. G. K. WESTERBRINK and J. GOUDSMIT, *Enzymologia* **10**, 146 (1941/42).
10. H. G. K. WESTERBRINK, *Proc. Fourth Int. Congr. Biochem.*, Vienna, Vol. XI, p. 72 (1959).
11. A. FUJITA, *Advanc. Enzymol.* **15**, 389 (1954).
12. A. VON MURALT, *Exp. Cell Res.*, Supp. 5, 72 (1958).
13. J. D. SPILLANE, *Biochemical Aspects of Neurological Disorders*, p. 17. Blackwell Scientific Publication, Oxford (1959).
14. C. A. ELVEHJEM, *J. Amer. Med. Ass.* **120**, 1388 (1942).
15. D. GLICK and W. ANTOPEL, *J. Pharmacol.* **65**, 389 (1939).
16. E. A. ZELLER, *Helv. Chem. Acta* **22**, 837 (1939).

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### Inhibition of dopamine β-oxidase by imipramine

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PREVIOUSLY we have reported that phenylethylamines and phenylpropylamines inhibit the conversion *in vitro* of dopamine to norepinephrine by dopamine β oxidase. The conversion of dopamine to norepinephrine may be the rate-limiting step in the biosynthesis of norepinephrine and, consequently, inhibitors of dopamine hydroxylation may be important therapeutic agents in lowering norepinephrine levels or increasing dopamine levels *in vivo*. The present report shows that imipramine (N-(γ-dimethylaminopropyl)-iminodibenzyl), a known anti-depressant agent, inhibits the conversion *in vitro* of dopamine to norepinephrine.

The enzyme dopamine β-oxidase was prepared by the method of E. Y. Levine *et al.*,[1] and the incubation was carried out by the procedure described in the previous paper.[2] At the end of the period of incubation, the solution was analyzed for the enzymically formed norepinephrine by two different experimental procedures. In the first procedure, dopamine-$C^{14}$ was used as a substrate, and after acetylation of the amines the enzymically formed norepinephrine-$C^{14}$ was separated from dopamine-$C^{14}$ by paper chromatography.[3] The amount of dopamine-$C^{14}$ which disappeared from the incubation mixture, as well as the amount of norepinephrine-$C^{14}$ formed in the incubation mixture, was calculated from the radioactivity of each of these compounds. In the second series, non-radioactive dopamine was used as a substrate and the enzymatically formed norepinephrine was determined by a modification of the fluoremetric method of von Euler and Floding.[4] The degree of inhibition rate of dopamine β oxidase was determined in both procedures by comparing the amount of norepinephrine formed in an incubation mixture which contained only the substrate and in an incubation mixture which contained imipramine and the substrate. Table 1 shows the effects of imipramine on the conversion of dopamine to norepinephrine. It is evident that when the concentration of inhibitor to substrate is 2:1 approximately 50 per cent inhibition of norepinephrine synthesis is observed.

Since inhibition of monoamine oxidase by imipramine was shown to be negligible[5] there is no basis for speculation along this line concerning its mode of action as a psychoenergizer. The present finding that imipramine inhibits dopamine β-oxidase may suggest that its pharmacological activity is a consequence of inhibition of norepinephrine synthesis which results in an increase of dopamine

TABLE 1. INHIBITION OF DOPAMINE TO NOREPINEPHRINE CONVERSION BY IMIPRAMINE*

| Expt. no.† | Dopamine added (μ moles) | Imipramine added (μ moles) | Norepinephrine formed (μ moles) | Dopamine disappearing (μ moles) |
|---|---|---|---|---|
| 1 | 0·4 | none | 0·12 ± 0·02 | 0·23 ± 0·05 |
| 1 | 0·4 | 2·00 | 0·07 ± 0·02 | 0·29 ± 0·05 |
| 2 | 4·00 | none | 1·46 ± 0·08 | not estimated |
| 2 | 4·00 | 4·00 | 1·43 ± 0·08 | not estimated |
| 2 | 4·00 | 8·00 | 1·00 ± 0·08 | not estimated |
| 2 | 4·00 | 12·00 | 0·75 ± 0·06 | not estimated |

* Figures represent averages of 3 experiments in each series.
† In Experiment 1, 0·02 ml of the standard enzyme solution was added to the incubation mixture and dopamine-$C^{14}$ was used as a substrate. In Experiment 2, 0·2 ml of the standard enzyme solution was added and non-radioactive dopamine was used as a substrate. The final volume of each incubation mixture was adjusted with 0·02 N phosphate buffer, pH 6·4, to 1·3 ml.

levels in various organs. Some preliminary studies with animals treated with imipramine have shown that in such animals the dopamine levels in various organs were higher than in untreated control animals, while the norepinephrine levels in the time period thus far studied were not altered significantly. Whether imipramine is a substrate for dopamine oxidase, as has been shown for phenylethylamines and phenylpropylamines,[6] is under investigation.

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*Department of Psychiatry and Neurology,*
*Neurochemistry Laboratory,*
*New York University College of Medicine,*
*New York, N.Y., U.S.A.*

M. GOLDSTEIN
J. F. CONTRERA

## REFERENCES

1. E. Y. LEVIN *et al.*, *J. Biol. Chem.*, **235**, 2080 (1960).
2. M. GOLDSTEIN and J. F. CONTRERA, *Biochem. Pharmacol.*, **7**, 77, (1961).
3. M. GOLDSTEIN *et al.*, *Proc. Soc. Exper. Biol. Med.*, **103**, 137 (1960).
4. U. S. VON EULER and I. FLODING, *Acta Physiol. Scand.*, **33**, 45 (1955).
5. P. GREENGARD, Private communication.
6. M. GOLDSTEIN *et al.*, *Experientia*, in press.