benzoate, a substance which uncouples phosphorylation from oxidation, has effects very similar to dinitrophenol in inhibiting the growth of influenza virus in tissue cultures and deembryonated eggs.

1. Eaton, M. D., *Arch. f. d. Gesamte Virusforsch.*, 1952, v5, 53.

2. Ackermann, W. W., *J. Exp. Med.*, 1953, v97, 315.

3. Eaton, M. D., and Perry, M. E., *J. Inf. Dis.*, 1953, in press.

4. Sheahan, M. M., Wilkinson, J. H., and Maclagan, N. F., *Biochem. J.*, 1951, v48, 188.

5. Dutoit, C. H., in *Phosphorous Metabolism*, edited by W. D. McElroy and Bentley Glass, 1952, v2, 597, Johns Hopkins Press, Baltimore, Md.

6. Lipmann, F., Personal communication.

7. Hoch, F. L., and Lipmann, F., *Fed. Proc.*, 1953, v12, 218.

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In vivo Inhibition of Monoamine Oxidase Studied with Radioactive Tyramine. (20541)

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In an earlier publication evidence was presented for the participation of monoamine oxidase in epinephrine metabolism and for inhibition of this enzyme *in vivo*(1). Epinephrine, because of the complexity of its metabolism(2) is not an adequate substrate for extended studies of monoamine oxidase *in vivo*. A more nearly ideal substrate, tyramine, a compound widely used for *in vitro* studies on monoamine oxidase, has therefore been synthesized in radioactive form. It is shown that with the exception of a small amount of conjugation, tyramine is metabolized by rats and mice only through monoamine oxidase action.

A simple method for testing *in vivo* inhibition of monoamine oxidase has been devised and applied to a number of compounds which not only have *in vitro* inhibitory power but also have pronounced pharmacological activity. Because of the relationship of some of these compounds to the sympathetic nervous system, a clarification of their effect on monoamine oxidase in intact animals is of considerable importance. It has been proposed by Gaddum and Kwiatkowski(3) that the sympathomimetic activity of ephedrine may be due to inhibition of monoamine oxidase and consequent protection of epinephrine from destruction. However, in the present study, no relationship is found between sympathomimetic activity and *in vivo* inhibitory power for monoamine oxidase.

Isotopic compounds. Tyramine, labeled with C^{14} in the α-position of the side chain was prepared by a small scale modification of the method of Johnson and Daschavsky (4). It involves thermal decarboxylation of DL-tyrosine, the latter being purchased in radioactive form from Tracerlab, Inc. The crude tyramine was purified by sublimation and converted to the picrate. The melting point of the radioactive tyramine picrate was 201°; Barger(5) reported 200°. For a non-isotopic sample synthesized by the same procedure, calculated C 45.9, H 3.85, N 15.3; found C 46.2, H 4.12, N 15.6. The compound showed only one radioactive spot on paper chromatograms. The specific activity was 3.3×10^6 c.p.m. per mg measured in a flow counter. Tyramine was converted to the hydrochloride before injection into animals.

Metabolism of tyramine. In order that radioactive tyramine be of maximum value in these experiments it was necessary to establish the extent to which monoamine oxidase is responsible for its metabolism. Previous workers had shown that in dogs, cats and

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rabbits(6) the major metabolite of tyramine was p-hydroxyphenylacetic acid, the expected end product of monoamine oxidase plus aldehyde oxidase action. This has now been confirmed in rats and mice. There is no evidence that any enzyme other than monoamine oxidase actually degrades the tyramine molecule, although there does appear to be a small degree of conjugation. Since p-hydroxyphenylacetic acid also appears partly in conjugated form, assays for inhibition were done on chromatograms of hydrolyzed urine. These showed only two spots corresponding to tyramine and p-hydroxyphenylacetic acid. Isotope dilution studies† of the combined urine of 5 rats injected subcutaneously with 0.5 μg C^{14} tyramine per g of body weight showed that 88% of the total radioactivity in the urine was present as p-hydroxyphenylacetic acid (mainly free) and 9% as tyramine (about half in free form). In the combined urine of 6 mice injected subcutaneously with 1 μg C^{14} tyramine per g of body weight, of the total radioactivity, 97% was present as p-hydroxyphenylacetic acid (mainly conjugated) and 12% tyramine (mainly free). Within the limits of error of the isotope dilution methods used, these two compounds can account for all the injected radioactivity. In another experiment the expired carbon dioxide of a mouse was found to contain, after 4 hr collection, 2% of the injected C^{14}.

Effect of various substances on monoamine oxidase using tyramine as a substrate. As a method for evaluating monoamine oxidase inhibitors *in vivo,* C^{14} tyramine was administered to mice pre-treated with the test substance and the amount of unmetabolized tyramine in the urine used as a measure of the degree of inhibition. This procedure appears to be valid since the hydrolyzed urine of mice which have been injected with C^{14} tyramine, contains in radioactive form only unmetabolized tyramine and the end-product of monoamine oxidase activity, p-hydroxyphenylacetic acid.

Mice were injected subcutaneously with the inhibitor and after 30 min. injected subcutaneously with 1 μg C^{14} tyramine per g of body weight. Urine was collected from each mouse at 30 min. intervals during a 4-hr period and immediately frozen. When the collection was complete, an approximately equal volume of 6 N hydrochloric acid was added to each urine and hydrolysis effected by heating for one hr in a steam bath. The hydrolyzed urine samples were evaporated to dryness from the frozen state, dissolved in a small amount of water, and paper chromatograms prepared using butanol 80 parts, ethanol 10 parts and ammonium hydroxide 30 parts. This system makes a clean separation of tyramine, Rf 0.9 and p-hydroxyphenylacetic acid, Rf 0.4. Only these two peaks were counted and corrected for background only; the rest of the paper contained negligible radioactivity. Per cent tyramine in the hydrolyzed urine was determined by dividing the c.p.m. in the tyramine peak by the sum of the c.p.m. in both peaks. This method is not intended to give an accurate determination of the absolute value of the tyramine content of the urine. It is designed only to give a base value, approximating the true value, from which deviations caused by inhibitors can be readily observed.

The test substances were chosen because of possible effect on monoamine oxidase. A brief description of each follows: *Ephedrine* inhibits monoamine oxidase *in vitro* using tyramine(7,8) or epinephrine(9) as substrates. It has powerful sympathomimetic properties and potentiates the action of epinephrine. It has been proposed(3) that ephedrine, and possibly other sympathomimetic amines, produce this effect by inhibiting monoamine oxidase and consequently prolonging the action of epinephrine. However, it is also possible that the action is directly on the same receptors acted upon by epinephrine. *Benzedrine and Paredrinol* are also potent sympathomimetic amines and *in vitro* inhibitors of monoamine oxidase(10,9). *Choline p-tolyl*

† In the isotope dilution studies urine was collected at 30 min. intervals for 4 hr. Carrier tyramine and p-hydroxyphenylacetic acid were added and separation effected by extracting the latter from acidic solution with ether. Both compounds were then recrystallized, using Norite each time, until constant activity was obtained. Tyramine was determined as the picrate; p-hydroxyphenylacetic acid as the free acid.

ether bromide has no obvious sympathomimetic effects. It is reported to be a much more potent *in vitro* monoamine oxidase inhibitor than benzedrine or ephedrine(11). It produces maximum potentiation of responses to epinephrine and norepinephrine in the cat at a level of 10 mg/kg.‡ *Marsilid* (1-isonicotinyl-2-isopropylhydrazine) has only mild sympathomimetic effects. It inhibits monoamine oxidase *in vitro*(12,13) and *in vivo* when tested using epinephrine as substrate (1). *Percaine* is a substitute for cocaine which is a monoamine oxidase inhibitor *in vitro*(8, 14). *Aramine* [levo-1 (m-hydroxyphenyl)-2-amino-1-propanol] is a potent pressor agent; Dapanone (3,4-dihydroxy-α-isopropylaminopropiophenone) is a potent bronchiodilator. They were generously supplied by Dr. Karl H. Beyer of Sharp and Dohme, Inc. *Thyroxin* has been reported to have some relationship to monoamine oxidase activity(15).

The concentrations used were sufficient to produce pronounced pharmacological effects. In some cases (Percaine, Paredrinol) the toxicity imposed a limit on the dose.

Results are shown in Table I.

Discussion. The data of Table I show that of all the substances tested, only Marsilid and choline p-tolyl ether are effective in inhibiting monoamine oxidase in the intact mouse. Other choline aryl ethers may be inhibitory but only the one compound of this class was examined in the present study.

Several of the compounds which inhibit monoamine oxidase *in vitro* were found to have no effect *in vivo*. This could be due to failure to reach the site of the enzyme because of rapid destruction or for reasons of permeability. It is also possible that *in vitro* preparations of monoamine oxidase have undergone some alteration of the active portion of the enzyme so that response to various substances differs from that in the body.

The powerful sympathomimetic amines, ephedrine, benzedrine and paredrinol showed no inhibitory effect. This strongly suggests that an explanation of their pharmacological activity must involve a mechanism unrelated to monoamine oxidase inhibition.

‡ Personal communication from Dr. Peter Hey, Department of Pharmacology, University of Leeds.

TABLE I. Effect of Various Compounds on Monoamine Oxidase Activity; C^{14} Tyramine Used as Substrate. All inhibitors injected subcutaneously; after 30 min. C^{14} tyramine injected subcutaneously at a level of 1.0 μg per g of body wt.

Test compound	Conc. of test compound, μg/g	% tyramine in urine
None		15
		16
		18
		22
Ephedrine	50	20
	100	21
Benzedrine (Amphetamine)	20	23
	40	22
Choline p-tolyl ether bromide	10	26
	25	28
	100	32
	100	33
	200	42
Marsilid phosphate	10	27
	25	32
	50	32
	100	31
	150	45
	300	47
	450	47
Percaine (cocaine substitute)	20	20
Aramine	50	14
Dapanone	50	19
Paredrinol sulfate (Veritol, p-hydroxy N-methyl benzedrine)	10	13
"	20	12
1-epinephrine (1 dose 30 min. before, 1 dose 30 min. after tyramine)	.5	19
Thyroxin (doses twice daily for 3 days and once on 4th day 1 hr before tyramine)	1.0	22

It is evident from Table I that none of the substances tested is capable of inhibiting monoamine oxidase to a very marked degree when tyramine is used as a substrate.

Summary. 1. Tyramine, labeled with C^{14} in the α-position of the side chain, has been synthesized for use as a substrate in studies of the *in vivo* inhibition of monoamine oxidase. 2. With the exception of a small degree of conjugation, no metabolic route for tyramine could be detected in intact rats and mice other than that due to monoamine oxidase action. 3. A technic for evaluating *in vivo* inhibitory effect on monoamine oxidase has been devised and a number of substances

tested. Only 2 active compounds were found; the common sympathomimetic amines were inactive.

1. Schayer, R. W., and Smiley, R. L., *J. Biol. Chem.*, 1953, v202, 425.
2. Schayer, R. W., Kennedy, J., and Smiley, R. L., *J. Biol. Chem.*, 1953, v202, 39.
3. Gaddum, J. H., and Kwiatkowski, H., *J. Physiol.*, 1938, v94, 87; 1939, v96, 385.
4. Johnson, T. B., and Daschavsky, P. G., *J. Biol. Chem.*, 1925, v62, 775.
5. Barger, G., *J. Chem. Soc.*, 1909, v95, 1128.
6. Ewins, A. J., and Laidlaw, P. P., *J. Physiol.*, 1910, v41, 28.
7. Blaschko, H., *J. Physiol.*, 1938, v93, 7.
8. Bernheim, F., and Bernheim, M. L. C., *J. Biol. Chem.*, 1925, v158, 425.
9. Heim, F., *Arch. f. Exp. Path. u. Pharmakol.*, 1947, v204, 520.
10. Mann, P. J. G., and Quastel, J. H., *Biochem. J.*, 1940, v34, 414.
11. Brown, B. G., and Hey, P., *J. Physiol.*, 1952, v118, 4.
12. Zeller, E. A., Barsky, J., Fouts, J. R., Kirchheimer, W. F., and Van Orden, L. S., *Experientia*, 1952, v8, 349.
13. Zeller, E. A., and Barsky, J., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 459.
14. Torda, C., *J. Pharm. and Exp. Therap.*, 1943, v78, 331.
15. Spinks, A., and Burn, J. H., *Brit. J. Pharmacol.*, 1952, v7, 93.

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Protection Afforded by Aqueous-Alcohol Solutions of Cortisone Against Dextran.* (20542)

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Attention has been called to the protection afforded by large daily doses of cortisone against the lethal anaphylactoid response of 24-hour adrenalectomized rats to intravenous infusions of the polysaccharide dextran(1). The animals were unequivocally shielded from shock but the procedure employed was slow and expensive since it required a total dosage of 60 mg of a microcrystalline suspension of cortisone (Merck's Cortone), administered over 5 days as a fore-treatment before giving the shock dose of dextran. It was considered desirable to devise a method whereby the dosage could be drastically reduced and the time necessary for adequate prophylaxis lessened without diminishing the effectiveness of the protection afforded. The following experiments demonstrate that, by using a dilute alcoholic solution of cortisone instead of the slowly absorbed microcrystalline suspension, the effective dose of the steroid which will give protection to more than 90% of animals can be reduced from 60 mg to 2 mg or less, and the time required for adequate fore-treatment shortened from 5 days to 6 hours. The simplicity of this modified procedure led the writers to adopt it as a test for screening compounds assumed to have cortisone-like activity.

Materials and methods. 138 virgin female rats averaging 133 g in weight were employed; when ready for use they were adrenalectomized and 24 hours later infused with dextran (Commercial Solvents Corp.; a sterile 6% solution in 0.9% saline); this material is used as a plasma expander for treatment of shock. The dextran was administered intravenously as a single injection, representing 7.5 mg/100 GBW (g of body weight), at the rate of one cc/minute. Rats with intact adrenals tolerate large doses of dextran by vein; however, the 24-hour adrenalectomized animal is extremely sensitive to the material and generally dies within 30 to 60 minutes after infusion(1). If the dextran is given intraperitoneally to intact rats in relatively huge doses, marked edema and erythema may appear(2,3). The striking

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