location of the phosphatase at the cell surface was reported for *E. coli*,[9] EAC cells,[8] and rat liver cells.[7] This study also suggests that MMPR-P was dephosphorylated to MMPR before (in ascites fluid) and/or during entry into cells, and then was rephosphorylated by the intracellular adenosine kinase. Cell lines resistant to MMPR, derived from a human epidermoid carcinoma and from EAC, have negligible levels of adenosine kinase.[4,6] This is further evidenced by the finding that no MMPR-P appeared in EAC-R2 cells after incubating them with the nucleotide (Table 1).

The survival of mice bearing EAC cells treated daily with either MMPR or MMPR-P (500 μmoles/kg) was identical. Mice bearing EAC-R2 cells did not respond to MMPR-P. Similar findings were also reported for arabinosyl cytosine monophosphate (ara-CMP) in leukemia L1210 and its subline resistant to ara-C.[13] This indicated that ara-CMP did not enter cells intact but was dephosphorylated prior to uptake.[13] The mouse survival data suggest that MMPR-P was dephosphorylated at an unfavorable rate, which offers no therapeutic advantage over MMPR. Therefore, the comparison of the rate of MMPR-P excretion with that of MMPR was not pursued.

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Amphetamine–tetrazolium reductase activity in brain

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The observation of Pugh and Quastel[1] that mescaline and amphetamine are attacked either feebly or not at all by brain tissue was confirmed by Bernheim and Bernheim[2] who demonstrated that rabbit liver oxidises mescaline very rapidly. Recently Seiler[3] showed that freshly prepared brain homogenate when incubated with mescaline sulfate at 37° for 18 hr catalysed the oxidation of mescaline by an enzyme which according to him is not diamine oxidase (DAO) but monoamine oxidase (MAO). Axelrod[4–7] and others[8,9] described deamination and hydroxylation of amphetamine, ephedrine and related compounds by liver tissues of various species, but to our knowledge it has not yet been demonstrated that brain tissue can metabolise amphetamine and ephedrine. Although liver possesses an active mescaline oxidase, there is much confusion regarding its nature. Various workers[10,11] suggested that it is different from MAO while Sourkes[12] postulated that mescaline oxidase and DAO are identical. Zeller *et al.*[13] and others[14,15] believe that mescaline is oxidised by MAO, DAO or both. Data are presented in this communication demonstrating the reduction of neo-tetrazolium chloride

(NTC) by rat and guinea-pig brain homogenates in the presence of *d*- and *l*-amphetamine, ephedrine and mescaline.

Whole brain homogenates of adult male albino rat (150–200 g) and guinea-pig (500–600 g) were prepared in isotonic sucrose solution. The standard reaction mixture contained 0·025 M phosphate buffers pH 7·5 or pH 7·0 for rat or guinea-pig brain homogenates respectively, 0·5 mg NTC, 0·01 M amphetamine, ephedrine or mescaline and 100 mg of freshly prepared brain homogenate in a final volume of 2 ml. All reagents excepting the homogenate were first added together and incubated at 37° for 5 min. The homogenate was also separately incubated under identical conditions and then an appropriate aliquot of the homogenate was added to the otherwise complete reaction mixture and the

TABLE 1. TETRAZOLIUM REDUCTASE ACTIVITY OF RAT AND GUINEA-PIG BRAIN HOMOGENATES

Additions	Rat brain homogenate		Guinea-pig brain homogenate	
	Aerobic incubation	*Anaerobic incubation	Aerobic incubation	*Anaerobic incubation
1. *d*-Amphetamine	14·9 ± 1·1	16·7 ± 3·5	16·7 ± 2·5	18·2 ± 2·4
2. *l*-Amphetamine	15·6 ± 2·8	17·8 ± 1·6	11·7 ± 1·5	14·0 ± 1·1
3. Ephedrine	11·6 ± 1·4	14·2 ± 2·1	6·7 ± 1·2	11·8 ± 0·7
4. Mescaline	12·9 ± 1·2	13·1 ± 2·1	Nil	12·7 ± 0·6

* NTC reduction in these experiments was measured after 15-min incubation. Values represent the mean of six experiments ± S.D. and expressed as μmoles of NTC-diformazan formed per 100 mg tissue. Other experimental details are given in the text.

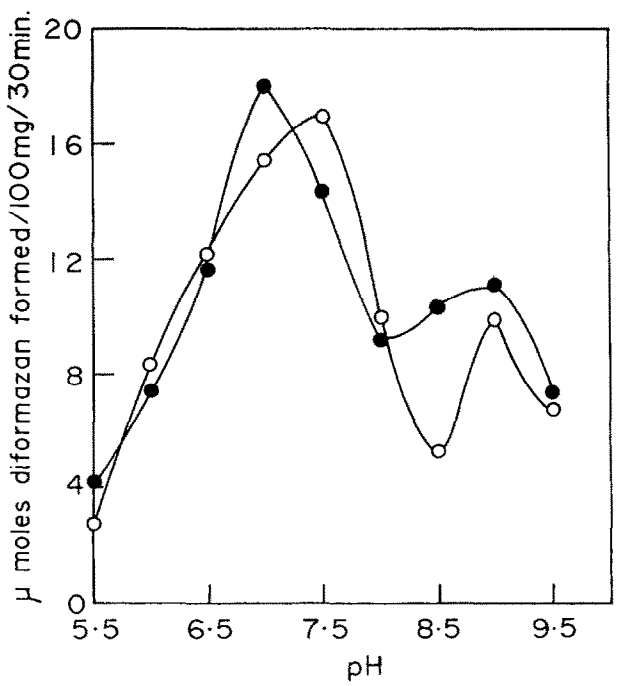

FIG 1. Influence of pH on NTC reduction by rat and guinea-pig brain homogenates.
○——○ rat brain homogenate with *l*-amphetamine.
●——● guinea-pig brain homogenate with *d*-amphetamine.

activity was determined after an incubation period of 30 min unless stated otherwise. The formazan was extracted and measured at 520 mμ according to the method of Lagnado and Sourkes.[16] When the inhibitors were added, they were first incubated with the homogenate for 10 min prior to addition of NTC and *d*-amphetamine. Air was used as the gas phase in aerobic experiments while anaerobic experiments were carried out *in vacuo* in Thunberg tubes.

Rat brain homogenates catalysed rapid reduction of NTC both aerobically and anaerobically in the presence of all the amines tested. When guinea-pig brain homogenate was used, formazan production increased both aerobically and anaerobically in the presence of amphetamine or ephedrine, but mescaline was only effective in the absence of air (Table 1). Among the different amines tested both the isomers of amphetamine were found to be most effective in producing tetrazolium reduction by rat and guinea-pig brain homogenates. Formazan production was markedly increased in all cases when air was excluded. Prior heating of brain homogenates for 5 min in a boiling water bath caused complete inactivation of tetrazolium reductase activity. There was also no extra production of ammonia or uptake of oxygen by respiring guinea-pig brain homogenates in the presence of *d*-amphetamine with or without NTC respectively confirming the previous findings of Pugh and Quastel.[1]

TABLE 2. EFFECT OF CERTAIN INHIBITORS ON *in vitro* NTC REDUCTION BY GUINEA-PIG BRAIN HOMOGENATE IN THE PRESENCE OF *d*-AMPHETAMINE

Inhibitors	Final concentrations of inhibitors (M)	Per cent inhibition
1. Potassium cyanide	2×10^{-2}	53
	2×10^{-3}	74
2. Semicarbazide	$1{\cdot}25 \times 10^{-2}$	nil
	$2{\cdot}5 \times 10^{-2}$	nil
3. Isoniazid	1×10^{-3}	nil
	1×10^{-4}	nil
4. Iproniazid	5×10^{-4}	43
	1×10^{-4}	nil
	5×10^{-5}	nil
5. Catron	5×10^{-4}	84
	5×10^{-5}	nil
6. Tranylcypromine	1×10^{-4}	nil
	1×10^{-5}	nil
7. Pargyline	1×10^{-4}	nil
	1×10^{-5}	nil

The pH–activity curves for NTC reduction by rat and guinea-pig brain homogenates in the presence of *l*-amphetamine and *d*-amphetamine respectively are shown in Fig. 1. NTC reductase system of rat brain exhibited two peaks at pH 7·5 and 9·0 pH while with guinea-pig brain the peaks were at pH 7·0 and pH 9·0. Seiler[3] also observed similar peaks in the pH-activity curve of mescaline oxidase of mouse brain homogenate. The effects of certain inhibitors on NTC reduction by guinea-pig brain homogenate in the presence of *d*-amphetamine are shown in Table 2. Semicarbazide, isoniazid, tranylcypromine and pargyline failed to inhibit formazan production whereas iproniazid and catron were effective only at high concentrations. On the other hand KCN-produced strong inhibition of NTC reduction. These effects indicate that amphetamine—NTC reductase system is possibly different from DAO, MAO or monoamine dehydrogenase described previously.[17]

The above data suggest the possibility that brain tissue possesses dehydrogenase systems capable of dehydrogenating amphetamine, ephedrine and mescaline similar to that observed in the case of dehydrogenation of biogenic amines.[16–18] The observation[19] that amphetamine—NTC reductase system of guinea-pig brain is localised in mitochondrial particles and needs supplementation of the soluble supernatant fraction containing a heat stable, dialysable co-factor or NADP for its full activity exclude the possibility of stimulation of endogenous formazan production in brain homogenates under the influence of these drugs.

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L-3,4-Dihydroxyphenylalanine-induced release of norepinephrine from the rat heart

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LARGE doses of L-dopa have been employed successfully in the symptomatic treatment of patients with Parkinson's disease for the last 3–4 years.[1–3] Some patients treated with L-dopa have experienced considerable interference with peripheral autonomic regulation. Orthostatic hypotension frequently has been reported and, less commonly, cardiac arrhythmias and hypertension have been noted.[1,3] Information is accumulating rapidly about the effects of L-dopa on brain amines.[4–6] Less is known, however, about the changes induced by L-dopa on the sympathetic innervation of organs outside the central nervous system. These studies were undertaken to explore the effects of L-dopa on norepinephrine (NE) metabolism at the sympathetic nerve endings in the heart.

Tracer doses of [^{3}H]NE, administered as an intravenous pulse, were utilized in this study. After intravenous administration [^{3}H]NE mixes with the endogenous NE pool and serves as a valid marker of changes in NE turnover.[7] Evidence is presented in support of the hypothesis that L-dopa, after conversion to dopamine, releases NE from the stores in the sympathetic nerve endings of the heart.

Material and Methods

All experiments were performed on female Sprague–Dawley rats, 150–180 g. The methods used were similar to those described previously.[8] DL-[7-^{3}H]NE (10–13 c/m-moles) was obtained from the New England Nuclear Corp. and purified prior to use by column chromatography with alumina. It was administered (25 μc/kg) to unanesthetized animals via the tail vein. L-Dopa, supplied as Laradopa