Histochemical amd biochemical effects of diethyldithiocarbamate on tissue catecholamines

SIR,—Recently diethyldithiocarbamate, an inhibitor of dopamine-β-oxidase, has been found to cause a decrease in noradrenaline and an increase in dopamine in rat ileum (Collins, 1965). The present study was planned to elucidate this effect further by means of a combined histochemical and biochemical approach.

Some male, albino, Sprague-Dawley rats (200–300 g) were treated with a single dose of 500 mg/kg s.c. of diethyldithiocarbamate (calculated as the sodium salt with 3 molecules of water of crystallisation) and killed at various intervals after the injection, while others received 2 doses of 500 mg/kg s.c., 7 and 3–4 hr before death. The brain, heart, submaxillary and adrenal glands, small intestine and femoral muscle were examined. Some animals were taken for the biochemical assay of dopamine (Carlsson & Waldeck, 1958; Carlsson & Lindqvist, 1962) and noradrenaline (Bertler, Carlsson & Rosengren, 1958). Others were taken for histochemical analysis (Falck, Hillarp, Thieme & Torp, 1962; Falck, 1962; Hillarp, Fuxe & Dahlström, 1965).

TABLE 1. NORADRENALINE (NA) AND DOPAMINE (DA) IN RAT HEART, BRAIN AND ILEUM AT VARIOUS INTERVALS AFTER SODIUM DIETHYLDITHIOCARBAMATE, 500 MG/KG S.C.

The values are means ± standard errors of the means, expressed in μg/g tissue.
Figures in brackets indicate number of experiments. Each experiment was performed on 2 or 4 pooled organs.

	Heart		Brain		Ileum	
Interval	NA	DA	NA	DA	NA	DA
Normal	0·77 (9) ± 0·029	0·02 (8) ± 0·010	0·41 (9) ± 0·017	0·64 (9) ± 0·028	0·25 (7) ± 0·017	0·03 (8) ± 0·007
2 hr	0·74 (1)	0·06 (1)	0·15 (1)	0·70 (1)	0·12 (1)	0·06 (1)
4 hr	0·72 (2) ± 0·020	0·09 (2) ± 0·040	0·14 (2) ± 0·050	0·77 (2) ± 0·070	0·12 (2) ± 0·010	0·08 (2) ± 0·020
6 hr	0·62 (3) ± 0·093	0·07 (3) ± 0·024	0·14 (3) ± 0·015	0·66 (3) ± 0·033	0·15 (3) ± 0·015	0·08 (3) ± 0·020
24 hr	0·96 (1)	0·12 (1)	0·29 (1)	0·72 (1)	0·17 (1)	0·04 (1)

After the single dose of diethyldithiocarbamate (500 mg/kg; preliminary observations show that much lower doses are active biochemically as well as pharmacologically) the animals appeared sedated but were easily aroused. Biochemical analysis of the brains revealed a decrease to about 30% of the normal value in noradrenaline 2–6 hr after injection (Table 1). After 24 hr the noradrenaline level was possibly still somewhat below normal. Dopamine did not change significantly. Histochemically no certain decrease in fluorescence intensity could be detected in central or peripheral catecholamine neurones at any interval studied. Biochemically a moderate decrease in noradrenaline was seen in the ileum but not in the heart. Dopamine showed an increase in both tissues, but levels sufficient to prove identity were hardly reached.

After two doses of diethyldithiocarbamate (500 mg/kg each) the animals were possibly somewhat more sedated than after a single dose. A marked depletion (to about 10% of normal) was obtained in the brain noradrenaline levels (Table 2), whereas the brain dopamine levels showed no significant change. In 3 experiments the brains were divided into hemispheres, striatum and stem; in the brain stem the dopamine levels were regularly (average 150%) higher in

the treated animals than in the controls; the same tendency was seen in the hemispheres, while no difference was seen in the striatum. The decrease in noradrenaline appeared to be the same in these different parts of the brain. Histochemically the various noradrenaline terminal systems of the brain were markedly depleted of fluorescent substances, whereas the catecholamine nerve terminals of the neostriatum, the tuberculum olfactorium, the nucleus accumbens, the median eminence, the nucleus amygdaloideus centralis and the dorsal part of the nucleus interstitialis striae terminalis showed normal fluorescence intensity. These areas have been found to contain mainly dopamine nerve terminals (see Fuxe, 1965; Carlsson, Dahlström, Fuxe & Hillarp, 1965). All the catecholamine nerve cell groups, however, showed normal fluorescence intensity after this treatment. The amine levels of the 5-hydroxytryptamine neurones were unaffected.

TABLE 2. EFFECT OF SODIUM DIETHYLDITHIOCARBAMATE (TWO SUBCUTANEOUS INJECTIONS OF 500 MG/KG 7 AND 4 HR BEFORE KILLING) ON RAT TISSUE CATECHOLAMINES

The values are means ± standard errors of the means. For the adrenals dopamine and the sum of adrenaline and noradrenaline are given in μg/2 adrenals. The other values are expressed in μg/g tissue.
Figures in brackets indicate number of experiments. Each experiment was performed on 2 or 4 pooled organs.

	Heart	Brain	Ileum	Femoral muscle	Adrenals
Noradrenaline					
Normal	0·77 (9) ± 0·029	0·41 (9) ± 0·017	0·25 (7) ± 0·017	0·12 (5) ± 0·014	28·7 (5) ± 3·390
Treated	0·80 (4) ± 0·049	0·05 (4) ± 0·011	0·10 (3) ± 0·025	0·07 (3) ± 0·006	18·4 (3) ± 2·322
Dopamine					
Normal	0·02 (8) ± 0·010	0·64 (9) ± 0·028	0·03 (8) ± 0·007	0·01 (5) ± 0·002	0·23 (4) ± 0·043
Treated	0·08 (4) ± 0·023	0·73 (4) ± 0·052	0·06 (4) ± 0·006	0·03 (3) ± 0·004	1·48 (3) ± 0·145

Biochemical analyses revealed a significant decrease in the noradrenaline levels of the ileum and femoral muscle, but not of the heart and adrenals. These effects were not large enough to be detected histochemically. The dopamine levels behaved as after a single dose; in the adrenals a considerable increase in dopamine was observed. In this case there is no doubt about the identity.

Histochemical observations on brains of rats treated with two doses of diethyldithiocarbamate as above and, in addition, nialamide (500 mg/kg intraperitoneally 6 hr before killing) revealed a normal fluorescence microscopic picture with normal fluorescence intensity in all parts of the catecholamine neurones of the brain. The controls receiving no nialamide showed marked noradrenaline depletion as described above. The nialamide-treated animals were less sedated than the controls.

The present data support the view that diethyldithiocarbamate inhibits dopamine-β-oxidase *in vivo*. The concomitant decrease in noradrenaline and increase in dopamine—except in dopamine neurones—supports this view. Although the percentage increase in dopamine after diethyldithiocarbamate treatment was probably large in the various noradrenaline-containing tissues examined, it reached levels which permitted identification only in the adrenals

and in the brain stem. We have not been able to confirm the high dopamine levels reported by Collins to occur even in normal ileum. We have examined various parts of normal rat ileum and obtained very low, hardly significant values for dopamine.

The present results reveal beyond doubt that a marked and selective depletion of the amine content of the brain noradrenaline nerve terminals occurred after treatment with diethyldithiocarbamate. The amine content of the central dopamine nerve terminals, on the other hand, was unchanged. By using diethyldithiocarbamate in this way it now seems possible to separate the dopamine and noradrenaline nerve terminals from each other. The histochemical experiments with diethyldithiocarbamate in combination with nialamide may be interpreted to show either, that noradrenaline is preserved by monoamine oxidase inhibition, or that it is replaced by dopamine. Biochemical studies are necessary to elucidate this point.

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