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DOPAMINE-β-HYDROXYLASE INHIBITION BY DIMETHYLDITHIOCARBAMATE AND RELATED COMPOUNDS

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Abstract—The effects of dimethyldithiocarbamate, disulfiram (tetraethylthiuram disulfide), diethyldithiocarbamate and ethyldithiocarbamate on dopamine-β-hydroxylase activity *in vivo* were determined. Dimethyldithiocarbamate and disulfiram inhibited the conversion of dopamine-^{14}C to norepinephrine-^{14}C in the heart and adrenals of the rat and hamster. Diethyldithiocarbamate and ethyldithiocarbamate did not appear to inhibit the enzyme in the hamster heart, but did in the hamster adrenals and rat heart and adrenals.

DOPAMINE-β-HYDROXYLASE[3,4-dihydroxyphenylethylamine, ascorbate: O_2 oxidoreductase (hydroxylating), EC1.14.2.1] is the enzyme which catalyzes the conversion of dopamine (DA) to norepinephrine (NE), the final step in the predominant pathway of NE biosynthesis.[1–4] The conversion of DA to NE has been demonstrated to occur in the adrenal by Leeper and Udenfriend,[3] in heart by Booker *et al.*,[5] in brain by Pisano *et al.*,[6] in spleen by Symchowicz and Korduba[7] and in other tissues rich in adrenergic endings.

Disulfiram (tetraethylthiuram disulfide) and diethyldithiocarbamate are potent inhibitors of dopamine-β-hydroxylase, which has been demonstrated both *in vivo* and *in vitro* by Goldstein *et al.*,[8] Musacchio *et al.*,[9] Collins,[10] and Goldstein and Nakajima.[11] It appears that disulfiram is active through its breakdown product, diethyldithiocarbamate, as shown by Goldstein *et al.*[12] At least one other related compound, phenylethyldithiocarbamate, is also known to be an inhibitor of dopamine-β-hydroxylase *in vivo*.[13]

This report describes the finding that dimethyldithiocarbamate and ethyldithiocarbamate, two compounds structurally related to disulfiram and diethyldithiocarbamate (Fig. 1), are inhibitors of dopamine-β-hydroxylase *in vivo*. Furthermore, the comparative inhibitory activities of these four structurally related compounds on dopamine-β-hydroxylase are described.

METHODS

Female Charles River albino rats weighing approximately 250 g were obtained from Canadian Breeding Laboratories (Laprairie, Quebec). Dimethyldithiocarbamate (City Chemical Co., New York), ethyldithiocarbamate (Eastman Organic), disulfiram (Ayerst Laboratories) and diethyldithiocarbamate (Eastman Organic) were injected (125 mg/kg, i.p.) in distilled water, except for disulfiram which was suspended in 1%

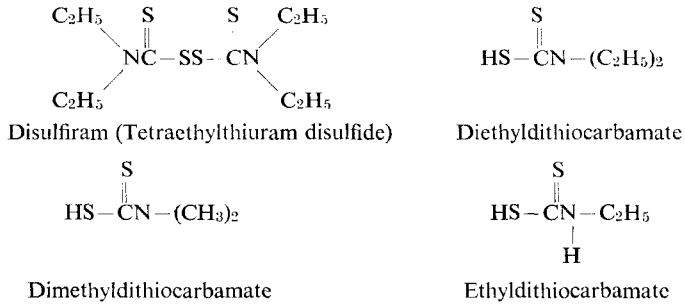

FIG. 1. Structural configuration of the four compounds studied.

carboxymethylcellulose. Control animals were injected with isotonic saline or 1% carboxymethylcellulose. The animals were sacrificed by decapitation 1, 6 and 24 hr after injection of the test compound. One-half hr before sacrifice, the animals were injected intramuscularly with $8{\cdot}88 \times 10^6$ dpm dopamine-1-^{14}C (32 mc/m-mole; Radiochemical Centre, Amersham, U.K.) in 0·25 ml of distilled water.

The assay utilized for the determination of the conversion of DA-^{14}C to NE-^{14}C was essentially that of Missala *et al.*[14] Immediately after sacrifice of the animals, the heart and adrenals were removed, wrapped individually (adrenals in pairs) in parafilm, frozen on dry ice, and stored in a freezer until homogenized. The tissues were cut finely (while frozen) and placed in a homogenizing vessel containing 5 ml ethanol–0·01% HCl and 3 mg ascorbic acid. They were homogenized in as short a time as possible, transferred to a cellulose nitrate centrifuge tube (Beckman Instrument Co.) with 5 ml ethanol–HCl and centrifuged at 100,000 *g* for 1 hr in a Spinco model L ultracentrifuge (Beckman Instrument Co.). The supernatant fluids were decanted into 15-ml graduated centrifuge tubes and evaporated under nitrogen to dryness. The samples were then taken up in 0·2 ml ethanol–HCl.

Approximately 150–200 μl of the sample was applied to a 2 cm wide, 48 cm long (running length) strip of phosphocellulose-81 ion-exchange chromatography paper (Whatman), and developed with butanol:acetic acid:water (4:1:1) for 48 hr. Radioactive NE and DA standards were routinely cochromatographed in all experiments. After development, the chromatograms were dried in a fume hood and cut into 1 cm long pieces and placed in scintillation vials for counting. Toluene phosphor scintillator [10 ml; 0·4% 2,5-diphenyloxazole and 0·005% 1,4-*bis*-(5-phenyloxazolyl)-benzene; Aldrich Chem. Company] was used in the determination of the amount of radioactivity in each strip piece. The peaks corresponding to DA and NE were totalled and the per cent conversion of DA determined as NE/(NE + DA) × 100. When the results are expressed as per cent NE of control they are calculated as:

$$\left(\frac{\text{NE}}{\text{NE} + \text{DA}}\right)_{\text{treated}} \Big/ \left(\frac{\text{NE}}{\text{NE} + \text{DA}}\right)_{\text{control}} \times 100.$$

For the hamster experiments, female Syrian Golden hamsters approximately 125 g in weight were obtained from the Canadian Breeding Laboratories. The procedure followed was as described for the rat, except that the hamsters were sacrificed 1 hr after the injection with DA-^{14}C.

In all experiments at least three animals were used; in the case of diethyldithiocarbamate in the hamster, a total of seven animals were used in two experiments.

RESULTS

Rats. From a series of time experiments (three animals/group) it was found that in the rat approximately 50 per cent of the radioactivity in the heart was present as NE-^{14}C 0·5 hr after the injection of the DA-^{14}C (Fig. 2). The same was found to be true in the adrenal. Thus, for subsequent studies it was decided to sacrifice the rats 0·5 hr after the DA injection.

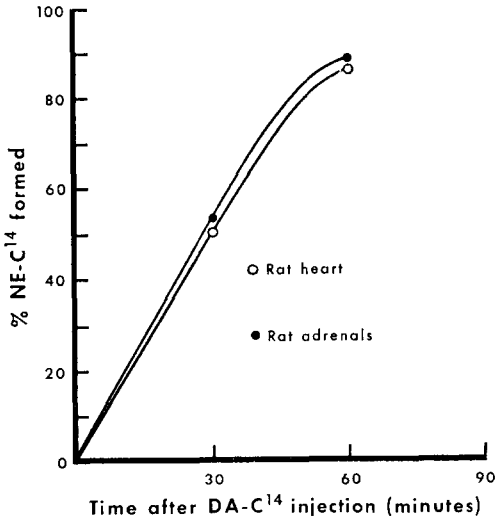

FIG. 2. Normal conversion of DA-^{14}C to NE-^{14}C in the rat. Animals (three per group) were injected with DA-^{14}C and sacrificed after 30 and 60 min. The S.E. was less than the radius of the point.

The effects of the drugs on the metabolism of DA-^{14}C in the rat tissues are shown in typical chromatographic patterns (Fig. 3) with dimethyldithiocarbamate (125 mg/kg, i.p.) in the rat heart being used for illustration.

In the rat, disulfiram (125 mg/kg, i.p.) caused essentially complete inhibition of DA-β-hydroxylase activity after 1 hr; the inhibition was marked even at 24 hr. Rats receiving dimethyldithiocarbamate, ethyldithiocarbamate or diethyldithiocarbamate all showed an initial complete inhibition of dopamine-β-hydroxylase; however, the enzymatic activity had returned to control levels 24 hr after injection. The degree of inhibition after dimethyldithiocarbamate, at 6 hr, was about the same as after ethyldithiocarbamate, but greater than that after diethyldithiocarbamate and less than that after disulfiram (Fig. 4). In all cases in the rat, the adrenals were affected to a much greater degree than was the heart, and inhibition was observed after each compound at 24 hr. (Also, in the adrenals similar results were obtained after diethyldithiocarbamate whether the injection vehicle utilized was carboxymethylcellulose or water.) The level of inhibition found after dimethyldithiocarbamate was in a similar relative relationship to the other compounds as that observed in the heart (Fig. 5).

Hamsters. In Fig. 6 is shown the rate of conversion of DA-^{14}C to NE-^{14}C in the heart and adrenals of the hamster utilizing three animals per group. It took approxi-

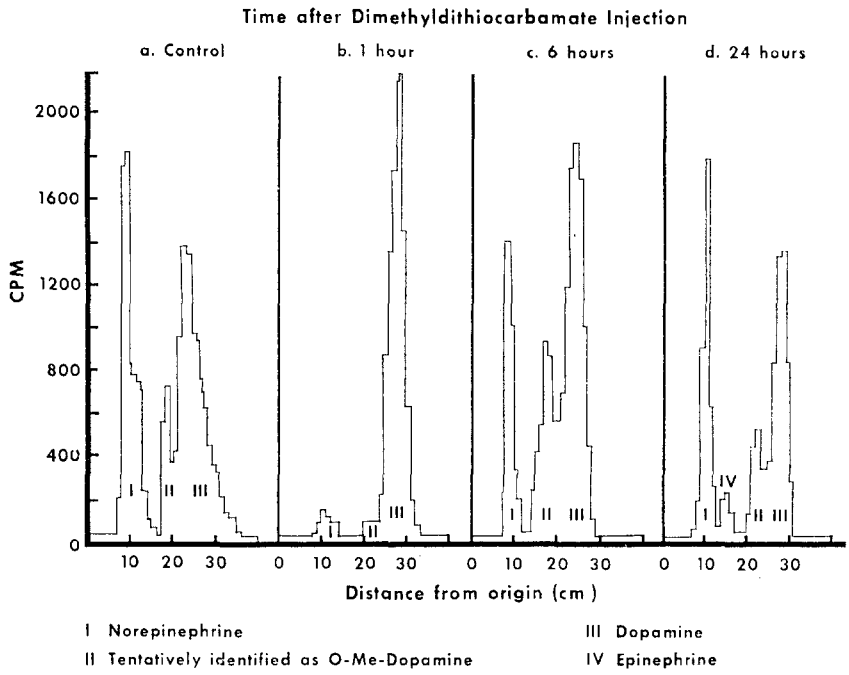

FIG. 3. Phosphocellulose-81 chromatograms obtained from heart extracts of rats treated with dimethyldithiocarbamate (125 mg/kg).

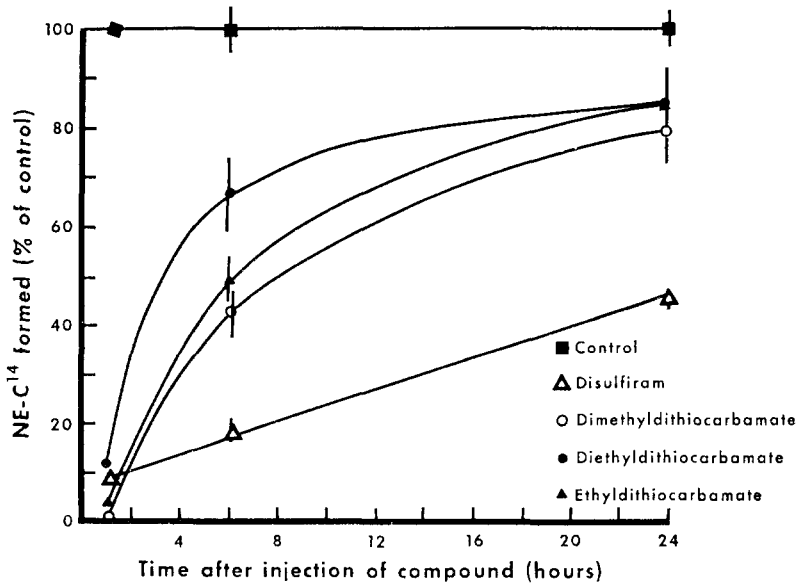

FIG. 4. Effects of dimethyldithiocarbamate and related compounds on the dopamine-β-hydroxylase activity of the rat heart. The compounds were injected at 125 mg/kg, i.p., and the animals sacrificed 1, 6 and 24 hr later. The animals were injected with DA-^{14}C 0·5 hr before sacrifice. Each point is the mean ± S.E. of at least three animals.

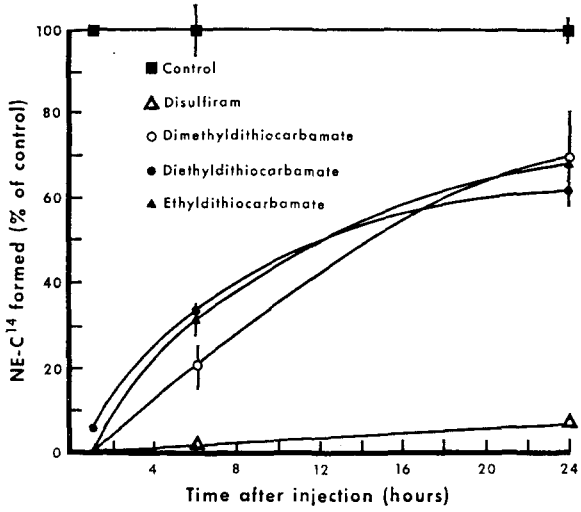

FIG. 5. Effects of dimethyldithiocarbamate and related compounds on the dopamine-β-hydroxylase activity of the adrenals of the rat. Compounds were injected at 125 mg/kg, i.p., and animals were sacrificed 1, 6 and 24 hr later. DA-^{14}C was injected 0·5 hr before sacrifice. Each point is the mean ± S.E. of at least three animals.

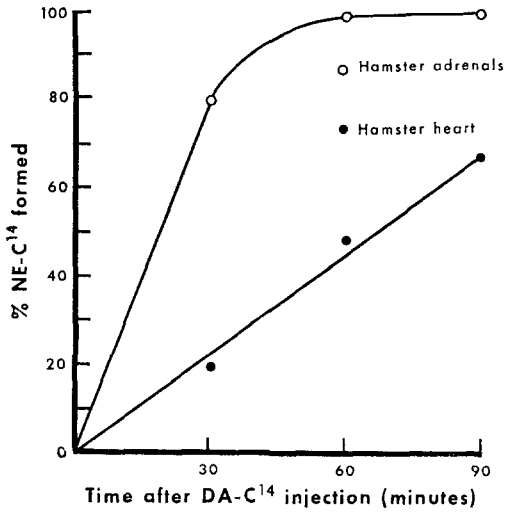

FIG. 6. Normal conversion of DA-^{14}C to NE-^{14}C in the hamster. Animals (three per group) were injected with DA-^{14}C and sacrificed 30, 60 and 90 min later. The S.E. was less than the radius of the point.

mately 60 min for the heart to reach the 50 per cent conversion level, whereas the adrenals exhibited 80 per cent conversion in 30 min.

When dimethyldithiocarbamate, disulfiram, diethyldithiocarbamate and ethyldithiocarbamate were injected at 125 mg/kg, i.p., in the hamster (Figs. 7, 8), large differences were observed among the four compounds. Dimethyldithiocarbamate and disulfiram caused complete inhibition of dopamine-β-hydroxylase in the heart

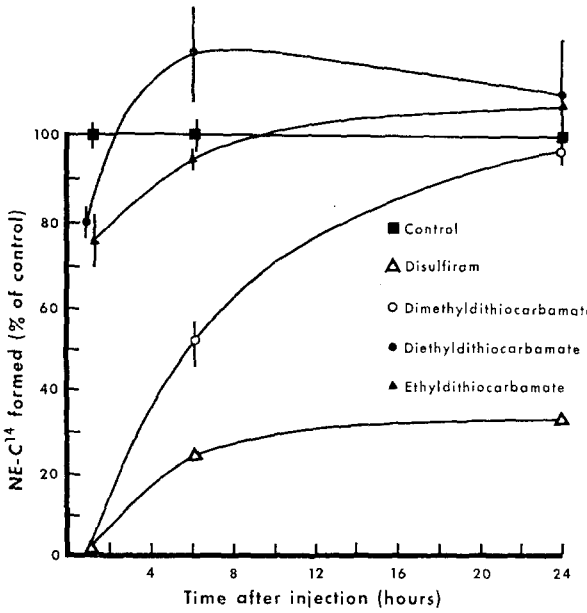

FIG. 7. Effects of dimethyldithiocarbamate and related compounds on the dopamine-β-hydroxylase activity in the hamster heart. Animals were injected with 125 mg/kg, i.p., of the drug and sacrificed 1, 6 and 24 hr later. DA-^{14}C was injected 1 hr before sacrifice. Each point is the mean ± S.E. of at least three animals.

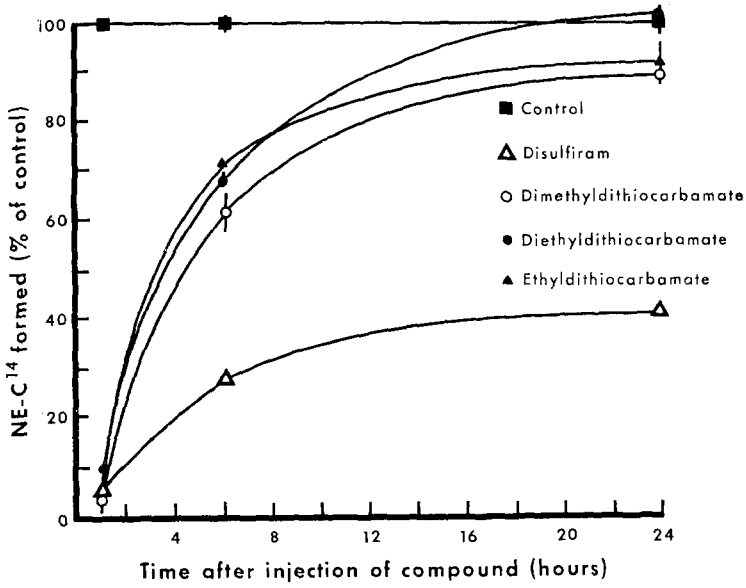

FIG. 8. Effects of dimethyldithiocarbamate and related compounds on the dopamine-β-hydroxylase activity of the hamster adrenals. The compounds were injected at a dose of 125 mg/kg, i.p., and the animals sacrificed 1, 6 and 24 hr later. One hr prior to sacrifice the animals were injected with DA-^{14}C. Each point is the mean ± S.E. of at least three animals.

after 1 hr. After 24 hr, the activity in the dimethyldithiocarbamate-treated animals had returned to the normal level, whereas in the disulfiram-treated animals there was still a marked inhibition (Fig. 7). In contrast to dimethyldithiocarbamate and disulfiram, both ethyldithiocarbamate and diethyldithiocarbamate gave very limited initial inhibition of the enzyme (26 and 19 per cent) and rapidly returned to control levels and above (128 per cent in the case of diethyldithiocarbamate at 6 hr).

In the adrenals each of the four compounds caused an initial complete inhibition of the enzyme (Fig. 8). Disulfiram after 24 hr still showed a large inhibition, whereas the other three compounds exhibited partial inhibitions at 6 hr and the enzyme activity had recovered to normal after 24 hr.

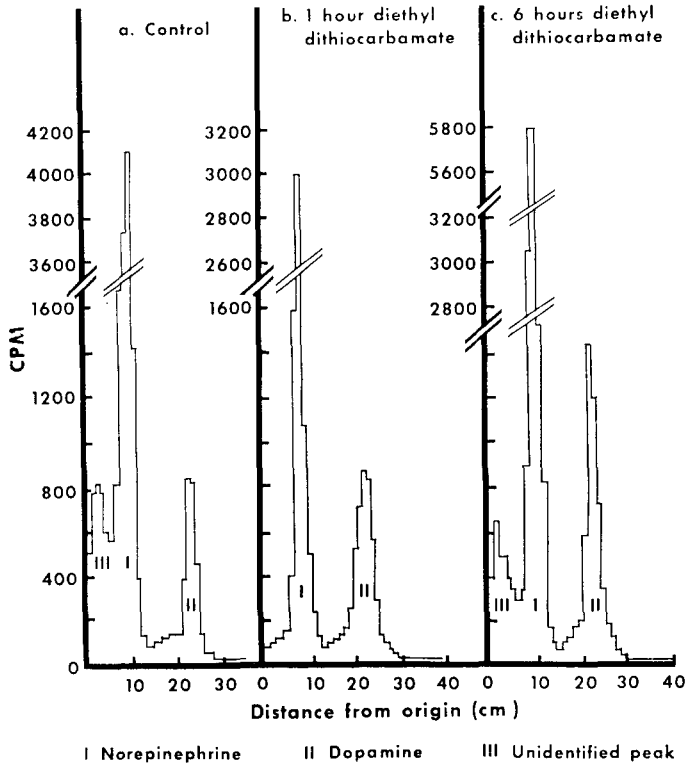

FIG. 9. Phosphocellulose-81 chromatograms of the hamster heart after diethyldithiocarbamate injection.

An additional peak appeared in chromatograms of the hearts of hamsters which did not receive any drugs (Fig. 9); the peak did not correspond to any of those in Fig. 3. The peak was not present in the diethyldithiocarbamate or ethyldithiocarbamate chromatograms at 1 hr, but reappeared (although to a lesser extent than in the controls) at 6 hr. Investigations are now in progress to determine the nature of this peak.

In both the rat and the hamster ethyldithiocarbamate was dissimilar to the other compounds tested in that it caused saliva secretion, severe diarrhoea and, in the case of the rat, a sweaty secretion on the tail. These effects all disappeared within an hour.

DISCUSSION

The effects of dimethyldithiocarbamate and the structurally related disulfiram (tetraethylthiuram disulfide), diethyldithiocarbamate and ethyldithiocarbamate on the conversion of dopamine-^{14}C to norepinephrine-^{14}C in tissues of the rat and hamster have been determined. In order to establish the conditions to be used for the studies of the effects of the compounds on the formation of NE-^{14}C, the normal rates of conversion in the hearts and adrenals of the rat and hamster were determined. Important differences were noted in this respect. In the rat, the rate of conversion of DA-^{14}C to NE-^{14}C is similar in the heart and adrenal, whereas in the hamster the conversion in the heart is slow and that in the hamster adrenal very rapid. Further, in comparison between the two species the conversion in the hamster heart is the slowest and in the hamster adrenal the most rapid. Of the various organs and species studied by Symchowicz *et al.*,[15] Symchowicz and Korduba,[7] and the present investigators, the conversion in the hamster adrenal appears to be the most rapid (Figs. 2 and 6).

The structurally related compounds are similar in that they all cause inhibition of the formation of NE-^{14}C from DA-^{14}C. However, significant differences are observed in their potency, duration of action, and tissue specificity. Disulfiram is the most effective inhibitor of the group in all of the tissues examined. Dimethyldithiocarbamate is more potent than either diethyldithiocarbamate or ethyldithiocarbamate in all tissues at 1 and 6 hr after injection. With respect to the relative potency observed, the report of Hald *et al.*[16] is of interest. In both the dithiocarbamate and thiuram disulfide series, an increase in chain length decreased the potency of the compound with respect to the LD_{50} in mice and the effect of alcohol metabolism in rabbits. In all cases the thiuram disulfide was more potent than the corresponding dithiocarbamate. Our enzymatic results suggest the same type of activity relationship.

With respect to the duration of activity, disulfiram exhibits a prolonged inhibition of dopamine-β-hydroxylase in both the heart and adrenal of the rat and hamster. However, the trend of recovery of activity after disulfiram in the hamster, in contrast to the rat, is similar in the heart and adrenal. The rate of recovery after dimethyldithiocarbamate is similar in the different tissues and species studied. The levels of conversion after diethyldithiocarbamate and ethyldithiocarbamate also rise in a similar fashion in the rat heart and adrenal and hamster adrenal; however, the hamster heart exhibits a striking difference. After diethyldithiocarbamate or ethyldithiocarbamate, only a slight initial inhibition of the activity is observed in the hamster heart and, in addition, the rate of conversion returns to normal within 6 hr; whereas, in rat heart and adrenals and hamster adrenals, all dithiocarbamate inhibitions are still observed at 6 hr. It is of interest that Goldstein *et al.*[8,12] reported that disulfiram markedly inhibits dopamine-β-hydroxylase, probably after reduction to form diethyldithiocarbamate both *in vivo* and *in vitro*. In the hamster heart (Fig. 7) the inhibition by diethyldithiocarbamate is very small at 1 hr and is above normal at 6 hr, whereas the inhibition by disulfiram is still very apparent after 24 hr; in all other cases

both compounds inhibit the enzyme. It is possible that the hamster heart is different with respect to its metabolic rates or patterns or both in comparison with the other tissues.

It is of importance to note that treatment with disulfiram at a dose higher than that employed here does not interfere with the uptake, storage, metabolism or release of DA or NE in the heart[8,9,17] of the rat. It has also been demonstrated that when dopamine-β-hydroxylase is inhibited by disulfiram the excess DA becomes bound in the tissues without being further metabolized.[8] Diethyldithiocarbamate, the reduction product of disulfiram, has been shown to be devoid of any ganglion blocking or adrenergic neurone blocking activity and it also does not exhibit any α- or β-receptor blocking activity.[18] Thus, it appears likely that both the decrease in NE-^{14}C formed from DA-^{14}C and the decrease in endogenous NE (as reported by others) caused by these compounds are due to inhibition of the dopamine-β-hydroxylase.

The experimental conditions used here are of importance in that this is the first time that the known potent inhibitors, disulfiram and diethyldithiocarbamate, have been compared in a time study at a single low dose in a normal rat. Symchowicz *et al.*[15] studied the effect of disulfiram on the incorporation and metabolism of DA-^{14}C in the hypertensive rat. At 125 mg/kg the results these authors obtained correlate well with the present findings. In all other reports (e.g. Jonsson,[13] Goldstein *et al.*,[8] Goldstein and Nakajima,[11] and Carlsson *et al.*[19]), the dose levels used were either higher than the ones in this report or else a series of injections was given, so that a comparable time study is unobtainable. Neither the effects of dimethyldithiocarbamate nor those of ethyldithiocarbamate on dopamine-β-hydroxylase have been reported previously.

Due to the previously discussed differences in the effects observed in the rat and hamster after administration of the four compounds, it appears that there is a species difference in the ability, and perhaps in the manner, by which these two species metabolize dimethyldithiocarbamate and structurally related compounds. Further, within the same species, in addition to the differences observed among the four compounds in the level of inhibition of dopamine-β-hydroxylase activity, differences have also been found in their effects on gonadotrophin secretion in the hamster. Lippmann* observed that disulfiram inhibited ovulation when given on proestrous whereas dimethyldithiocarbamate did not. Dimethyldithiocarbamate did, however, block ovulation when given on the second day of diestrous. Diethyldithiocarbamate, in contrast, did not block ovulation when given on either day. Differences were also found in the effects of the three compounds on the duration of diestrous in the hamster.

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