Inhibition by disulfiram of the accelerated turnover of catecholamines in the adrenal glands of exercised rats

SIR,—Disulfiram is a potent inhibitor *in vitro* and *in vivo* of dopamine β-hydroxylase, the enzyme that catalyzes the final step in noradrenaline synthesis (Goldstein, Anagnoste & Lauber, 1964; Musacchio, Kopin & Snyder, 1964; Goldstein, Lauber & McKereghan, 1965). Disulfiram lowers heart and brain noradrenaline levels to a greater extent in cold-exposed rats than in rats kept at room temperature (Goldstein & Nakajima, 1966). This supports the view that a more rapid turnover of catecholamines occurs in brain and heart of cold-exposed animals. The accelerated catecholamine turnover in brain and heart can also be blocked with α-methyltyrosine (Gordon, Spector & others, 1966). α-Methyltyrosine inhibits tyrosine hydroxylase, the first and possibly rate-limiting enzyme in catecholamine synthesis. Using this inhibitor, an increased turnover of adrenal adrenaline was found to occur in rats forced to exercise (Gordon & others, 1966).

The results of Goldstein and Nakajima and of Gordon & others, suggest that disulfiram would lower adrenaline levels in the adrenal glands of exercised rats, although inhibitors of catecholamine synthesis produce little change in adrenal adrenaline in normal rats because of its slow turnover. We report the results of our experiments with disulfiram on the level of adrenaline in the adrenals of exercised rats.

Male Sprague-Dawley rats of approximately 160 g were injected intraperitoneally with disulfiram in a suspension of 5% acacia. The rats were then placed in a wood and screenwire drum (39 cm diameter) that rotated at approximately 8 rev/min, requiring the animals to run to remain upright. After 3 hr, the rats were decapitated and the adrenal glands quickly removed and frozen on dry ice. Adrenaline levels were measured fluorimetrically (Shore & Olin, 1958), the oxidation step being made at pH 5 so that noradrenaline was also included. Experiments in our laboratory have shown that noradrenaline comprises less than 10% of the total adrenal catecholamine as measured by this method; hence, the results are expressed as adrenaline. They are shown as means and standard errors of determinations in Table 1.

TABLE 1. ADRENALINE AND NORADRENALINE LEVELS IN THE ADRENAL GLANDS OF RATS (4 rats/group)

Treatment	Adrenaline + noradrenaline	
	μg/mg tissue	μg/adrenal pair
Control	1·05 ± 0·13	22·6 ± 0·5
Disulfiram	1·15 ± 0·08	21·9 ± 0·4
Exercise	1·10 ± 0·08	21·2 ± 1·0
Disulfiram + exercise	0·47 ± 0·06*	9·9 ● 0·5**

* Differed from saline controls, $P < 0.01$.
** Differed from saline controls, $P < 0.001$.

Disulfiram, at the 200 mg/kg dose, did not produce any effect in control rats during the 3 hr period studied. Likewise, exercise alone had no effect. Gordon & others (1966) found slight depletion of adrenal adrenaline after 3 hr of exercise in rats but our exercise conditions are probably less severe. However, the combination of exercise and disulfiram treatment caused a fall in adrenal adrenaline of more than 50% (Table 1).

These results are in agreement with those of Gordon & others (1966) in showing an increased turnover of adrenal catecholamines in exercised rats and demonstrate that this accelerated synthesis can be blocked by inhibiting an enzyme

other than tyrosine hydroxylase. Our results also show that disulfiram can inhibit noradrenaline and adrenaline synthesis in the adrenal glands as it does in the heart and brain.

In another experiment, rats were treated with 400 mg/kg of disulfiram intraperitoneally—the dose used by Goldstein & Nakajima to block brain and heart catecholamine turnover in cold-exposed rats. None of the treated rats survived the 3 hr of forced exercise. Goldstein & Nakajima mentioned that disulfiram-treated animals were sensitive to cold exposure. Our experiment shows that they are also very sensitive to exercise. Doses up to 1 g/kg of disulfiram were not lethal for up to three days in rats that were not exercised.

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Increased uptake of noradrenaline in the rat submaxillary gland during sympathetic nerve stimulation

SIR,—Uptake of noradrenaline in various organs innervated by adrenergic nerves has been studied extensively by a number of authors. However, it is not known whether this uptake mechanism is a stationary process or if there are factors which regulate the uptake of the neurotransmitter according to physiological state of the adrenergic neurons. This kind of information is important from the viewpoint that the reuptake of noradrenaline is regarded as the major pathway of the nerve impulse-released amine (Hertting & Axelrod, 1961; Rosell, Kopin & Axelrod, 1963; Gillespie & Kirpekar, 1965) and that the need for replacement of noradrenaline in the nerve endings as well as the amount of noradrenaline released would be increased when nerve activity increases. Gillis (1963) has shown that stimulation of the cardio-accelerator nerves caused an increased retention of noradrenaline by cat atria but not by ventricles while Blakeley & Brown (1964) observed a decreased uptake by the cat perfused spleen during nerve stimulation. The present paper shows that the uptake mechanism is enhanced on increasing the sympathetic activity in the submaxillary gland of rats.

Long Evans rats of either sex, weighing 250–300 g, were anaesthetized with intravenous injection of chloralose, 60 mg/kg. The rats were prepared for the stimulation of the sympathetic nerve to submaxillary gland according to the procedure described by Sedvall & Kopin (1967). The cervical sympathetic trunk and vagus nerve of one side were ligated and freed from the carotid artery and cut at the level of the clavicle. The vagus nerve was freed from the superior cervical ganglion and cut to interrupt afferent vagal impulses. The sympathetic nerve trunk was stimulated intermittently (10 sec each min) with rectangular pulses (5–7 V, 5 msec duration) at a frequency of 20/sec. The effectiveness of electrical stimulation was confirmed by salivation and wide opening of the eye