A new principle for the fluorimetric determination of α-methylnoradrenaline

SIR,—α-Methyldopa, a drug widely used in the treatment of hypertension, is converted *in vivo* to α-methylnoradrenaline which displaces the endogenous noradrenaline (Carlsson & Lindqvist, 1962). The absolute configuration of this α-methylnoradrenaline appears to be 1*R*:2*S*, i.e., the (−)-*erythro* form (Lindmar & Muscholl, 1965). Although there are methods for the determination of α-methylnoradrenaline (Lindmar & Muscholl, 1965; Schümann, Grobecker & Schmidt, 1965), there is a need for a more sensitive method. It is also of importance to make a differential estimation of α-methylnoradrenaline and noradrenaline since these two compounds are not easily separated by cation-exchange chromatography.

Recently it has been shown that the *erythro*-α-methylnoradrenaline can be converted to the *threo* form in an acid medium (Hallhagen & Waldeck, 1968). Further it has been observed by Muscholl (personal communication) that the *threo* form treated according to the trihydroxyindole-method (Bertler, Carlsson & Rosengren, 1958) yields a fluorescence of the same order of magnitude as noradrenaline whereas the fluorescence obtained from *erythro*-α-methylnoradrenaline under the same experimental conditions is much lower.

In the present investigation 0·2–2·0 μg of the *erythro* form was added to 5 ml of 2N hydrochloric acid and the samples were then heated in a boiling water bath under a reflux condenser for 30 min. Non-heated samples were run in parallel. The samples were then neutralized to pH 6·5 by 5N potassium carbonate. One ml of the neutral sample was taken for fluorimetric determination (Bertler & others 1958). Fig. 1 shows that there is a constant ratio between the fluorescence and the concentration and that the method is sensitive down to 20–30 ng/ml sample.

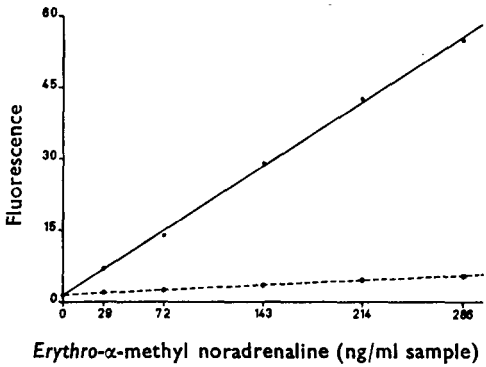

FIG. 1. Fluorescence intensity at various concentrations of *erythro*- α-methylnoradrenaline when treated according to the trihydroxyindole method with and without previous boiling in an acid medium (see text). Activating and fluorescence peaks: 400 and 510 mμ, respectively. —•— Boiled samples. — —•— — Unboiled samples.

In another experiment equal amounts (2 μg) of *threo*- and *erythro* α-methylnoradrenaline and noradrenaline were treated as described above. The fluorescence intensities of the samples were then compared. The mean of the unboiled samples of the *threo*-form was set to 100 (Table 1). When not boiled, *threo*-α-methylnoradrenaline showed a fluorescence intensity several times higher than its diastereoisomer. The boiling procedure, however, caused about 30%

decrease of the fluorescence obtained. In contrast, *erythro*-α-methylnoradrenaline after boiling gained a fluorescence of the same intensity as the boiled *threo* form. It thus appears that under the present conditions an equilibrium between the two diastereoisomers is reached. The fluorescence from noradrenaline was but slightly reduced after boiling in an acid medium (Table 1). So it would be possible to make differential estimations of *erythro*-α-methylnoradrenaline and noradrenaline.

TABLE 1. THE RELATIVE FLUORESCENCE INTENSITY OF *threo*-α-METHYLNORADRENALINE (α-Me-NA), *erythro*-α-Me-NA AND NORADRENALINE (NA) WHEN TREATED ACCORDING TO THE TRIHYDROXYINDOLE METHOD WITH AND WITHOUT PREVIOUS BOILING IN AN ACID MEDIUM (SEE TEXT). ACTIVATING AND FLUORESCENCE PEAKS: 400 AND 510 mμ, RESPECTIVELY

	threo-α-Me-NA	*erythro*-α-Me-NA	NA
Unboiled ..	100 ± 5	6 ± 1	103 ± 5
Boiled	69 ± 1	69 ± 2	92 ± 2

Mean ± s.e.m. of 3–5 determinations.

Experiments are now in progress to apply this principle on eluates from tissue extracts.

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Inhibition of ethanol-induced glycogenolysis in brain and liver by adrenergic β-blockade

SIR,—Ethanol is known to increase lipolysis in adipose tissue (Brodie, Butler & others, 1961; Estler & Ammon, 1967; Ammon, Estler & others, 1966) and glycogenolysis in brain and liver (Ammon, Estler & Heim, 1965; Ammon, Estler & Heim, 1966; Estler & Ammon, 1965). The increased lipolysis is supposed to be due to the action of catecholamines which are released from the adrenal medulla by ethanol (Klingman & McC. Goodall, 1957; Abelin, Herren & Berli, 1958; Perman, 1961; Wartburg, Berli & Aebi, 1961) because it can be prevented by adrenalectomy (Mallov & Gierke, 1957), α-adrenergic blocking agents (Brodie & others, 1961) and β-adrenergic blocking agents (Estler & Ammon, 1967). Since catecholamines increase glycogenolysis by stimulating the adenylcyclase system, which activates not only the hormone-sensitive lipase in adipose tissue but also phosphorylase, we examined whether the glycogenolytic action of ethanol in brain and liver is mediated by catecholamines which