Gas chromatographic evaluation of 5,5-dimethyl-2,4-oxazolidinedione (DMO) for determination of intracellular pH

The DMO technique is an effective way of calculating the total body and skeletal muscle intracellular pH (Longhini, Gallitelli & others, 1968; Schloerb & Grantham, 1965; Irvine & Dow, 1966; Constant, 1967). DMO evaluation is usually made using plasma, muscle and urine by the Waddell and Butler ultraviolet spectrophotometric method (Butler, 1953; Waddell & Butler, 1957, 1959), unless [2-^{14}C]5,5-dimethyl-2,4-oxazolidinedione is used (Schloerb & Grantham, 1965; Irvine & Dow, 1966).

In the gas-liquid chromatographic method DMO is first extracted, according to the Waddell & Butler technique (Butler, 1953; Waddell & Butler, 1957, 1959), the final solution from this method, i.e. in 0·05M borate buffer, pH 9, being acidified by 5N HCl to pH 1–1·5 and then extracted by peroxide-free ethyl ether four times. The ethyl ether extracts are dried over Na_2SO_4 and evaporated at 35° in a flow of nitrogen and the residue chromatographed (10% butandiol succinate on Gas-chrom Q 90–100 mesh, 2 m × 2 mm i.d. stainless steel, column temperature 213°, 20 ml N_2/min, DMO retention time relative to that of the internal standard, 5-methyl-2,4-oxazolidinedione, 0·64; detector: flame ionization 130°; injector: 265°. Both compounds are synthesized according to Stoughton (1941).

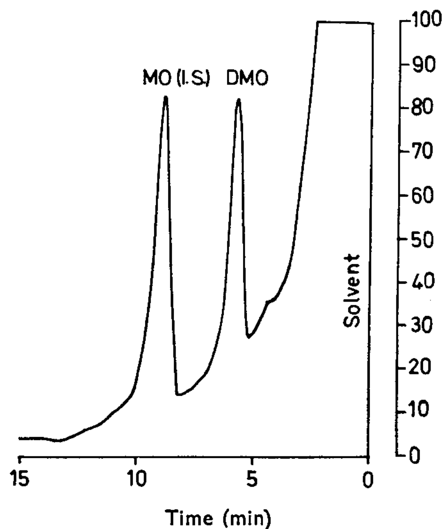

FIG. 1. Gas-chromatogram of DMO from human muscle sample. Analytical details are given in the text.

The tests on DMO value in blank, plasma, urine and skeletal muscle homogenates are in agreement with those obtained by us using the Waddell & Butler method. The chromatographic method, however, is sensitive (about 1 μg) and specific (Fig. 1) enough to enable DMO evaluation on human muscle samples obtained by needle-biopsy. This avoids the use of the expensive [2-^{14}C]5,5-dimethyl-2,4-oxazolidinedione.

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Is there a simple explanation for the sensitization to histamine produced by adrenergic β-receptor antagonists?

The high resistance of mice to the lethal effects of histamine can be reduced by *Bacillus pertussis* vaccine (Parfentjev & Goodline, 1948; Munoz & Bergman, 1966) or by adrenergic β-receptor antagonists (Fishel, Szentivanyl & Talamage, 1962; Townley, Trapani & Szentivanyl, 1967; Bergman & Munoz, 1968). On this basis, Fishel, Szentivanyl & Talamage (1964) have argued that the lethal effects of histamine in mice are usually antagonized by the simultaneous release of large amounts of catecholamines; when *B. pertussis* vaccine or other drugs block the adrenergic β-receptors, lethality is believed to have been increased by unbalanced α-receptor stimulation. This explanation is vulnerable because it has also been shown that histamine lethality is enhanced by adrenalectomy (Halpern & Wood, 1950) when a reduction in both α- and β-receptor stimulation would be expected, and that the enhanced lethality produced by a β-receptor antagonist can be reversed by a large dose of adrenaline (Bergman & Munoz, 1966, 1968) when even greater α-receptor stimulation would be expected. There is, of course, a simpler explanation which would resolve these difficulties. It is conceivable that histamine produces death in mice by acute bronchoconstriction and that this action is usually attenuated by the bronchodilator action of histamine-released catecholamines. Before attempting to evaluate this hypothesis directly, it seemed important to assess the potential effects of the non-specific actions of adrenergic β-receptor antagonists. To do this, the effects of both (+)- and (−)-isomers of D-(−)-2-isopropylamino-1-(*p*-nitrophenyl)ethanol (INPEA) (Almirante & Murmann, 1966) have been studied.

Differences in sensitivity to histamine due to environmental conditions and strain differences are known to exist. For this investigation, therefore, 2 random-bred strains (NMRI and AP-1) and 5 inbred strains (C3H/He/Sel, CE/Sel, DBA/2/Sel, C57L/Sel, C57Bl/10/Sel) were used with the aim of finding the most suitable strain. All the animals, including the inbred strains, come from our breeding station where they were kept solely on a diet of Rieper/MT pellets and deionized water. The environmental conditions in the laboratory were the same as in the animal quarters (25° ± 0·3). The animals had been fasted for 18 h before testing. To measure the sensitivity to histamine, groups of 10 adult male mice of each strain were given, intraperitoneally, doses of histamine HCl in saline corresponding to 15, 60 and 600 mg/kg histamine base. D-(−)-INPEA, L-(+)-INPEA and propranolol were injected intravenously at various doses in 0·2 ml saline at the rate of 0·01 ml/s, 15 min before the histamine challenge. Mice sensitive to histamine showed sedation, cyanosis, defeacation, unsteady gait and respiratory distress. Many of these animals convulsed and died within 5–20 min of the challenge injection. Only the 24 h toxicity value of histamine was estimated.

All the strains used showed the usual high resistance to the lethal effects of histamine and different animals in any strain varied greatly, both in the effects produced by