Radiochemical assay of catecholamines in blood plasma

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At present, the most promising approach to the assay of the very small quantities of the three catecholamines, known to be present in normal blood plasma, is the use of a suitable enzyme that allows the formation of identifiable radioactively labelled products. Passon & Peuler (1973) used the method of Engelman, Portnoy & Lovenberg (1968), in which a preparation of the enzyme catechol-O-methyl transferase enables the introduction of a radioactively labelled methyl group into the catecholamine molecule, and were able to carry out the reaction directly in small volumes of blood plasma. Fry, House & Sharman (1974) estimated the content of the catecholamines in the salivary gland of the cockroach after separation of such radioactive compounds, as their acetylated derivatives, by paper chromatography. This method of paper chromatographic separation has been preferred in the assay to be demonstrated since it allows the complete separation of acetylated, radioactive metanephrine, normetanephrine and methoxytyramine to be made with little difficulty. The acetylation of the methoxy-amines increases their stability so that there is little or no loss during chromatographic separation. We feel that there are fewer hazards in handling the paper chromatograms than with other methods of separation since the radioactive regions are simply cut out and eluted in the scintillation vial.

The demonstration will show the method used for the simultaneous estimation of noradrenaline, adrenaline and dopamine in the same sample of blood plasma and in small pieces of tissue. The reproduceability and sensitivity of the method will be illustrated and some results which have been obtained in studying widely different problems concerning catecholamines will be presented to demonstrate the suitability of the method for the screening of clinical material.

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A method for the micro-injection into mammalian muscle fibres of procion brilliant red H3BN

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A variety of techniques for the preparation and use of micropipettes filled with Procion dyes have been described (see Kater & Nicholson, 1973). The present method is simple and deviates little from the method used in our laboratories for the preparation of conventional recording microelectrodes.

Procion Brilliant Red H3BN, when obtained from source (I.C.I. Limited, Dyestuffs Division), is contaminated with a water insoluble de-dusting agent (Stead, 1973). This agent was first removed by rinsing with acetone. A solution of the dye (4% w/v) was then prepared by dissolving this partially purified product in distilled de-ionized water and filtering the solution through a 0.45 μm millipore filter. Micropipettes were pulled from 2 mm bore pyrex glass tubing. They were filled by boiling under reduced pressure with industrial methylated spirit or absolute ethanol, transferred to distilled water for 5 minutes, and then immersed overnight in a dye solution. Pipettes thus filled with Procion Brilliant Red had tip resistances of 20-30 MΩ and tip potentials less than 10 mV. Conventional microelectrodes filled with 3M KCl prepared by a similar procedure had tip resistances of 5-15 MΩ and tip potentials less than 10 mV. Dye-filled pipettes prepared in this manner and stored in Procion Brilliant Red

solution would usually pass current and eject dye up to two weeks after their manufacture but fresh solutions and electrodes were generally prepared once per week. Pipettes filled with the dye were capable of passing direct currents and square wave pulses of either polarity to magnitudes greater than 200 nA. Dye was ejected by anodal current. When a pipette was inserted into a muscle fibre a suitable rate of dye injection was effected by currents of 20-80 nA. Direct currents of 40 nA were normally used. Larger currents often caused blockage of the pipette due to coagulation of dye at the tip. A noticeable 'mark' could be seen in the muscle fibre within twenty seconds. Rapid fixation was necessary since the dye appears to diffuse from the point of injection within 5-10 minutes. For histological examination, cold Calcium Formol fixation (4°C for 4 h) results in low background fluorescence and the marked fibre is easily detected under a Fluorescence microscope (excitation 380 nm, emission 540 nm). For electron microscopic examination, the usual glutaraldehyde/osmium fixative is unsuitable as the former induces intense background fluorescence and the latter quenches all fluorescence completely. Of other fixatives tested, one comprising 0.8% glutaraldehyde 5% paraformaldehyde buffered to pH 7.2 has been used with some success.

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The use of halothane-induced sleeping time as an index of central nervous system excitability

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Sleeping time following an i.p. injection of a central nervous system (CNS) depressant drug e.g. hexobarbitone or pentobarbitone has often been used as an index of CNS excitability, particularly in drug tolerance studies. However, we have reported previously that a change in the rate of drug metabolism tends to mask any change in sensitivity of the CNS (Stevenson & Turnbull, 1970) and more useful information on the sensitivity of the brain to barbiturate may be obtained by determining the duration of anaesthesia following an intracerebroventricular injection of pentobarbitone (Stevenson & Turnbull, 1974). However, this latter method is unsuited to the study of change in CNS sensitivity occurring with time. We have therefore explored the usefulness of the measurement of the duration of anaesthesia produced by carefully controlled exposure to halothane.

The gas flow from an anaesthetic apparatus, incorporating a Fluotec Mark 2 vaporizer, was passed through a perspex box of dimensions 35 x 15 x 10 cm in which up to six 200 g rats could be placed. Oxygen containing 5% halothane was passed through the box at a flow rate of 2 litres min^{-1} for 3 min and the rats were exposed to this mixture for a further 5 min after switching off the gas flow. The animals were then removed from the box, placed on cotton wool and the time taken to regain the righting reflex was determined for each animal using a stopwatch. A sleeping time of between 3 min and 7 min, depending on the time of day at which animals were exposed to halothane, was obtained.

Pretreatment of animals with a CNS stimulant (amphetamine 1 mg kg^{-1} for 10 min) or depressant (sodium pentobarbitone 5 mg kg^{-1} for 5 min) shortened (2.58 ±0.18 (3) (± s.e.m.) min) or lengthened (9.18 ± 0.10 (3) min) the sleeping time respectively compared with saline pretreated rats (6.85 ± 0.17 (4) minutes).

Induction or inhibition of hepatic drug-metabolizing enzyme activity had no effect on the halothane-induced sleeping time (phenobarbitone pretreated 5.38 ± 0.20 (4); SKF 525A (2-diethylaminoethyl-2,2-diphenylvalerate HCl) pretreated 5.67 ± 0.26 (4); control 5.25 ± 0.10 (5) minutes).