A method for the measurement of therapeutic levels of (+)-amphetamine in human plasma

The absence of a specific and sensitive method for the measurement of therapeutic levels of unchanged and radioactively unlabelled (+)-amphetamine in human plasma has limited kinetic studies to investigations of urinary excretion (Beckett & Rowland, 1965a; Beckett, Tucker & Moffat, 1967). I present here an improved procedure based on the gas-chromatographic technique of Cartoni & Stefano (1963), subsequently developed by Beckett & Rowland (1965b), which has now enabled plasma levels to be estimated.

Procedure. Plasma (10 ml) was made alkaline by the addition of 1 ml N NaOH and extracted by gentle shaking with 10 ml and 5 ml aliquots of diethyl ether (distilled over Na wire to remove oxide contaminants) for 5 min in a 3-dimensional mechanical shaker (Desaga A.-G., Heidelberg). Vigorous shaking resulted in the formation of emulsions. After centrifuging at 4000 rev/min, the ether extracts were combined and stored in a glass-stoppered centrifuge tube (15 ml) at −5° to freeze out dissolved aqueous phase containing plasma constituents which would interfere with the subsequent detection. Drying with anhydrous Na_2SO_4 caused appreciable losses due to adsorption. The organic phase was decanted and 1 ml of an ethereal solution of *NN*-dimethylaniline (0·04 μg/ml) added as the internal standard. Transfer of the bases into 0·5 ml of 0·1N H_2SO_4 (ether-washed) was accomplished by passing a steady stream of N_2 through the acid phase to disperse the layers and give accompanying solvent evaporation. After washing the acid phase with diethyl ether (4 ml), this was basified by the addition of 0·2 ml of N NaOH (ether-washed) and shaken for 1 min with diethyl ether (2 ml) on a Fisons "Whirlimixer". On separation in the centrifuge, the ether layer was transferred to a special tube for controlled evaporation to 50μl with a slow stream of N_2 (18°). This consists of a 15 ml centrifuge tube drawn out at the bottom into a sealed narrow bore (1 inch long) with an approximate capacity of 100 μl (Laboratory Glassware Manufacturers, Beckenham, Kent).

Mild conditions are essential for evaporation of the solvent. If evaporation to dryness occurs, 80% or more of the amphetamine and a large proportion of the internal standard are volatilized. The final extracts are stored at −5° and removed immediately before analysis: this precipitates the aqueous layer and prevents evaporation of the organic phase.

Suitable peaks for (+)-amphetamine on the chromatogram recordings resulted when the columns were injected with 2–4 μl of the concentrate (Fig. 1). To calculate the concentration of (+)-amphetamine, a linear reference curve was constructed by plotting the ratio of the peak areas (height × width at half height) for (+)-amphetamine and *NN*-dimethylaniline over the concentration range 10–80 ng/ml of plasma.

Recoveries over this range were 75 ± 7%. Amphetamine is known to be bound to plasma proteins to the extent of approximately 15% (Axelrod, 1954). Comparison of the recovery of (+)-amphetamine obtained from plasma containing 10 ng/ml with that from an aqueous solution of the same concentration, indicated that both free and protein-bound forms of the drug were extracted *in toto* by the above procedure. This would infer that losses in recovery of (+)-amphetamine are in the main a consequence of its volatility.

A Series 104 model 24 chromatograph (W. G. Pye), equipped with dual flame ionization detectors, was used. The signal was recorded on a Honeywell −0·1 to +0·1 mV recorder. The column support was an 80–100 mesh acid-washed dimethyldichlorosilane-treated Chromosorb G, coated with 5% KOH and 15% Carbowax 6000. This was packed into silanized glass columns (1·5 m long × 4 mm internal diameter),

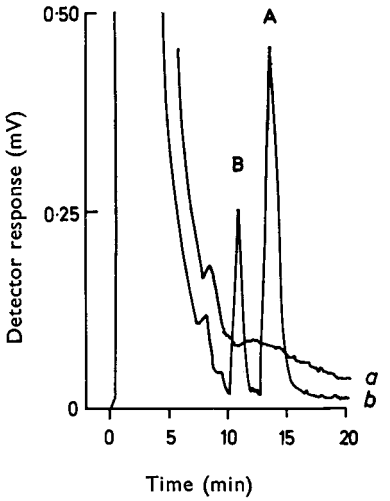

FIG. 1. The curve *a* represents an ether extract of plasma without (+)-amphetamine. Curve *b* represents an ether extract of plasma (3 μl injection) containing 40 ng of (+)-amphetamine (A) plus *NN*-dimethylaniline (B) as internal standard. An attenuation ($\times$ 10) equivalent to a current of 10^{-11}A and a chart speed of 10 inches/h were used.

which were conditioned on the chromatograph for 24 h at 180° before use. The columns were maintained at 165° with the injection port at 215°. Inlet pressures for H_2, N_2 and O_2 were 20, 50 and 30 lb/inch^2 respectively. Under these conditions, flow rates for H_2, N_2 and O_2 were 27, 27 and 325 ml/min repsectively. Replacement of compressed air by O_2 (Jones & Green, 1966) gave an 80% improvement in sensitivity, but to prevent the detectors from burning out, the high flow rate used was found to be essential. On the recordings, (+)-amphetamine was located by means of its relative retention time in comparison with *NN*-dimethylaniline. (+)-Amphetamine was characterized by observing the shift in retention time on transforming the drug into its acetone-derivative (Brochmann-Hanssen & Svendson, 1962). Losses of (+)-amphetamine by adsorption onto the solid phase of the column were minimized by presaturation of the active adsorption sites before each set of determinations. This was achieved by injecting an ethereal solution of nicotine (1 μg/ml) repeatedly until a constant base line was maintained (nicotine retention time 31·4 min). The use of (+)-amphetamine for this purpose may lead to erroneous results due to its displacement from the column sites by water contaminating the final extract. Before the injection of each sample, sufficient time was allowed for nicotine to be completely eluted from the column, since this can interfere in the determination of amphetamine in blood taken from smokers. Human subjects given 10–15 mg of (+)-amphetamine sulphate had maximum blood levels of 40–50 ng/ml after 1½–2 h, falling to 2 ng/ml (the lowest limit of detection) after 8–10 h.

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Serosal transfer of glucose during peristalsis

We have recently described a method (Gwee & Yeoh, 1968) for comparing the serosal appearance of a substance under "quiescent" and "peristaltic" conditions in the same piece of intestine. A solution of the substance is perfused through the lumen of the intestine at a constant pressure and peristaltic contractions may be initiated by causing the intestine to distend slightly. We now report the serosal transfer of glucose.

The salt solution used in the preliminary isolation and mounting of intestinal segments and also to bathe the serosal surface of the intestine (serosal fluid), contained only a minimal quantity of glucose (0·01 g/litre); the electrolyte composition (g/litre) was as follows: NaCl 6·92, KCl 0·353, $CaCl_2$ 0·282, $MgSO_4$ 0·142, KH_2PO_4 0·161, $NaHCO_3$ 1·0. The fluid used to perfuse the lumen (mucosal fluid) had the same electrolyte composition but the glucose concentration was increased to 3·6 g/litre.

After an initial rest period of 15 min and thereafter at 30 min intervals, the serosal fluid was sampled to determine the glucose concentration by the method of Nelson (1944). An experiment consisted of four 30 min periods of alternating quiescent and peristaltic conditions. Mean values for the change in serosal glucose concentration for 30 min periods gave the following results.

In 15 experiments the rate at which glucose appeared in the serosal fluid was $56 \pm 5{\cdot}1$ (s.e.) μg/cm intestine h^{-1} with the intestine quiescent and $135 \pm 8{\cdot}4$ with the intestine undergoing peristaltic contractions ($P < 0{\cdot}001$).

These results are consistent with the view that the serosal glucose is mainly transferred from the lumen and is not endogenous, since the rate of appearance of glucose on the serosal aspect varied with its concentration in the mucosal fluid, and the rate of appearance declined only slightly over the 2 h period of the experiments. Also, experiments with [^{14}C] labelled glucose in guinea-pig small intestine showed similar "transfer" rates for glucose estimated chemically, and for glucose estimated by its radioactive content (Yeoh & Lee, 1968).

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