A direct method for the isolation of catecholamines in plasma

An isolation method for catecholamines has been developed in which plasma, without prior manipulation, is passed directly over a small column of an ion exchange resin and the catecholamines are eluted as borate chelates which are suitable for immediate analysis by the trihydroxyindole methods. The entire procedure is performed at pH 4·0–7·5 thus avoiding possible hydrolysis of labile conjugates of the catecholamines. Furthermore, dopa is completely separated from the catecholamines and cannot interfere with their measurement. The method appears to be especially suited for the measurement of the elevated plasma concentrations of dopamine resulting from the administration of L-dopa.

The details for the preparation and elution of the resin column have been previously described as applied to the analysis of tissue samples (Minard & Grant, 1972), and they are applicable with only minor modifications to the analysis of plasma. In brief, a sample of plasma (heparinized and contained sodium metabisulphite at 0·5 mg ml^{-1}) at its natural pH of 7·0–7·5 was passed over a column of the weak cation exchange resin BR–70 (0·65 × 3·0 cm; 100–200 mesh; Bio-Rad Laboratories) which had been adjusted to pH 6·0 and was then equilibrated with 10 ml of 0·4 M sodium phosphate buffer at pH 6·0 immediately before use. The resin was contained in a 0·65 × 10 cm glass tube which was sealed to a 2·3 × 15 cm test tube that served as a reservoir for the water and boric acid which were used in the elution. The sample was washed through the column with three portions of 10 ml of water which were discarded, and catecholamines were eluted with 7·0 ml (3·0 ml + 4·0 ml) of a 2% aqueous solution of boric acid which has a natural pH of 4·0. Without further treatment, the eluate was analysed for dopamine by the iodine-trihydroxyindole method (Welch & Welch, 1969) with wavelengths of 326/390 nm (activation/emission), and for radioactivity by adding 3·0 ml of eluate to 10 ml of Aquasol for liquid scintillation counting. A 0·5 ml volume of an antioxidant solution (containing both ascorbic acid and disodium ethylenediamine-tetraacetic acid at a concentration of 3 mg ml^{-1}) was added to the sample volume and to all other solutions (10, 30, or 7 ml) passed over the column. The total time for the sample and other solutions to pass through the column was approximately 1½ h.

Emission spectra of the developed fluorescence in the borate eluates from plasma samples were determined with an activation wavelength of 326 nm, and uncorrected wavelengths of 326/390 nm (activation/emission) were found to be suitable for the

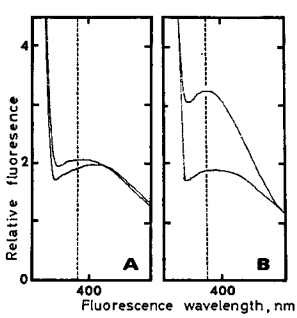

FIG. 1. Emission spectra of boric acid eluates and reversed blanks from human plasma after I_2-trihydroxyindole reaction when activated at 326 nm. Dashed line is at 390 nm. A: null addition of dopamine B: 0·05 μg ml^{-1} of dopamine added to plasma.

measurement of dopamine isolated from plasma (Fig. 1). The amount of light scattered at 326 nm was intense and it resulted perhaps from lipid particles adsorbed to the resin column. Although scatter never appeared to be a problem, heparinized blood was usually centrifuged at 10 000 *g* for 10 min to separate the plasma.

[^{14}C]Catecholamines (12 000 d. min^{-1}; 0·05 μg) were added to 1,3,6,10 or 15 ml volumes of human plasma pooled from three volunteers. These were chromatographed and the recoveries of ^{14}C-activity in the boric acid eluates were measured. The recoveries (% $\pm$ s.e.m.) of [^{14}C]amines were: dopamine 100·5 $\pm$ 1·18, noradrenaline 99·8 $\pm$ 0·95 and adrenaline 97·2 $\pm$ 1·83 (n=5 in all cases). Elution of the amines by boric acid is quantitative (Minard & Grant, 1972), and thus a lowered recovery of radioactivity would reflect a lessened retention of amine on the column. It is evident that up to 15 ml of human plasma can be passed over the column with complete retention of the three catecholamines. The per cent recoveries were corrected for the purity (85–95%) of the [^{14}C]amines which was determined by their chromatography in 5·0 ml of 0·9% saline solution.

Six graded quantities of dopamine, ranging from 0·007 to 0·15 μg ml^{-1}, were added to 5·0 ml volumes of pooled human plasma from three volunteers and these were chromatographed as described. A linear recovery of dopamine, ranging from 70–81%, was obtained. The lower limit of reasonable precision for the method, including the fluorimetric measurement, is approximately 0·01 μg dopamine ml^{-1} of plasma, but this sensitivity will vary because it depends upon technique and the purity of reagents.

Dopamine was also added in quantities of 0·02 and 0·10 μg ml^{-1} to duplicate 5·0 ml samples of plasma from 10 individuals who had fasted for 9 h, recoveries of 75 and 72% of theoretical were obtained together with the expected ratio of values.

Recoveries of dopamine were determined from 5·0 ml samples of pooled human plasma to which dopamine had been added at a level of 0·057 μg ml^{-1} and to which L-dopa was added in amounts ranging from 0 to 10 μg ml^{-1}. The recoveries for the six test samples averaged 93 $\pm$ 3·9% of that from the sample without added dopa, which demonstrates that dopa is not retained by the weak cation exchange resin and passes quantitatively through the column.

It is evident that the described method allows a reproducible isolation of the catecholamines in plasma (similar results were obtained with canine plasma) as a solution which is suitable for immediate analysis by the iodine trihydroxyindole procedure or liquid scintillation counting. It is a much less laborious method, in regard to the number of manipulations, than that involving separation on columns of alumina (Anton & Sayre, 1962).

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