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The use of Sephadex G-25 for the separation of catecholamines from plasma

The catecholamines adrenalin and noradrenalin have been separated from human plasma by adsorption on alumina[1,2] or on the cation-exchange resin Dowex-50 after precipitation of the serum proteins with perchloric acid[3,4]. Since recoveries of catecholamines from plasma by these methods were variable, the use of the dextran gel, Sephadex (Pharmacia, Uppsala, Sweden) has been investigated to remove the serum proteins.

15 g of dry Sephadex G-25 was prepared in a water-swollen particulate form, and used as a column, 2 cm diameter, 25 cm long, according to the method of FLODIN[5]. The water regain was 2.5 g of water per gram of dry material. The bed was stabilised over several hours with 0.01 M acetic acid before use. The void volume of the column, determined by the method of BENNICH[6], was 30 ml. The maximum volume of plasma which could be added to this column to give a satisfactory separation of catecholamines from plasma proteins was 20 ml.

The elution volumes for the catecholamines and plasma proteins were determined by passing the following solutions separately through the column: 2 ml adrenalin solution (1 mg/ml in 0.01 N HCl), 5 ml normal plasma (catecholamine content < 0.5 μg/l), and 5 ml plasma containing 2 mg of added adrenalin. The eluant was 0.01 M acetic acid. Fractions of 3 ml were collected, and the absorbancy at 280 mμ determined. The elution diagrams in Fig. 1 show that whilst the adrenalin (Fractions 17–41) and plasma proteins (Fractions 6–16) are effectively separated, a compound in the plasma absorbing at 280 mμ elutes with the adrenalin. Since this compound is partially retained by the gel, its molecular weight must be less than 3500. It did not interfere with the estimation of the catecholamines in the eluate by a modification of the fluorimetric trihydroxyindole method of LUND[1].

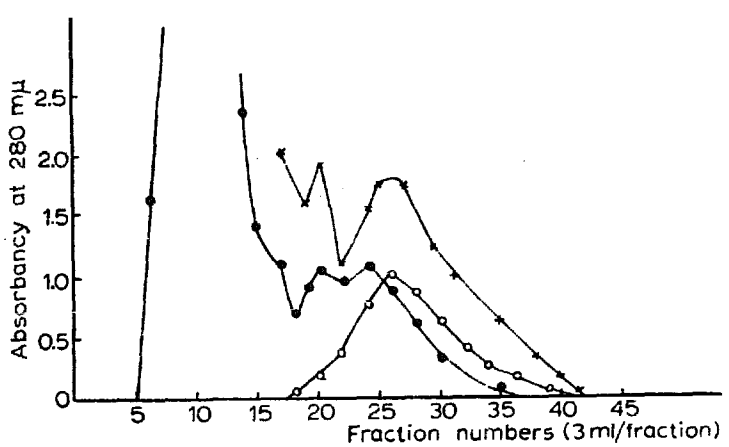

Fig. 1 Elution of adrenalin and plasma proteins from Sephadex G-25, by 0.01 M acetic acid. O—O, elution of 2 mg adrenalin; ●—●, elution of plasma proteins from 5 ml normal plasma; ×—×, elution of plasma proteins and adrenalin from 5 ml plasma containing 2 mg adrenalin.

Table I shows the recovery of added adrenalin from plasma samples to be at least 94%. The Sephadex column was regenerated by washing with 0.01 M acetic acid for about 2 h.

For estimation of the catecholamine content of normal and pathological plasma,

concentration of the eluate from the Sephadex column (75 ml) was necessary. This was effected by adsorption of the catecholamines on Dowex-50, by a modification of the method of BERTLER *et al.*[3], a procedure which also largely removed fluorescent impurities. The sodium form of Dowex-50 was prepared by washing with 50 ml 2 N HCl, 50 ml 1 N HCl–ethanol (1:1), 10 ml glass-distilled water, 20 ml 1 N sodium acetate buffer (pH 6.0) and 5 ml glass-distilled water. The eluate from the Sephadex column (pH 4.4) was shaken with 300 mg of the sodium form of the resin for approx. 20 min. The resin was collected by centrifugation, and washed with 50 ml of glass-distilled water. The catecholamines were eluted with 21 ml of 1 N HCl–ethanol (1:1) in 3-ml aliquots. The total eluate was reduced in volume to less than 8 ml in an evacuated desiccator, before estimation of the catecholamines.

TABLE I

PERCENTAGE RECOVERIES OF ADRENALIN ADDED TO 10 ml PLASMA SEPARATED ON SEPHADEX G-25

Adrenalin added (mg)	*Adrenalin recovered (mg)*	*Recovery (%)*
2	1.93	96.5
1.75	1.7	97
1.5	1.39	94
1.0	0.98	98

TABLE II

CATECHOLAMINES ADDED TO 15 ml OF PLASMA SEPARATED ON SEPHADEX G-25 AND DOWEX-50

Added		*Recovered*	
Adrenalin (µg)	*Noradrenalin (µg)*	*Adrenalin (µg)*	*Noradrenalin (µg)*
0.5	0.0	0.46	0.0
0.2	0.5	0.175	0.43
0.2	0.1	0.18	0.112
0.1	0.3	0.095	0.277
0.0	0.2	0.0	0.181

Recoveries of adrenalin and noradrenalin added to 15 ml of plasma by the overall procedure are shown in Table II. This method appears to give significantly greater and more reproducible recoveries than earlier methods.

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