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Note

A convenient system for the separation of some steroids on Sephadex LH-20

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This report describes how several of the major corticosteroids and some other biologically important steroids can be rapidly separated on Sephadex LH-20 columns, using methylene chloride as the eluting solvent. Sephadex LH-20 chromatography has been found to be particularly useful for the preliminary separation of steroids prior to radioimmunoassay and competitive protein binding assay[1–3]. In considering the problem of separating aldosterone and corticosterone, prior to radioimmunoassay of aldosterone in rat plasma, we were unable to find a system suitable for this purpose. Although a system has been reported for the rapid separation of these steroids on Sephadex LH-20, the columns require tedious preparation prior to use[4]. The single solvent system described below provides a fast chromatographic step combined with the minimum of column preparation.

EXPERIMENTAL

Approximately 4–5 g of Sephadex LH-20 gel (Pharmacia, Uppsala, Sweden) was swollen in methylene chloride overnight. Chromatography was carried out in glass columns (I.D. 1 cm), fitted with Teflon stopcocks. A small glass bead (diameter 0.5 cm) was used as the bed support. A piece of nylon gauze was placed on top of the column to prevent the gel from floating in the solvent. The gauze was held in place by a short piece of glass tubing.

Labelled steroids were transferred onto the column with three washings (each 0.1 ml) of methylene chloride. The eluting solvent was run onto the Sephadex gel from a burette clamped into position above the column. Fractions of 1 ml were collected into scintillation vials and evaporated to dryness. The radioactivity was measured by liquid scintillation counting. Recovery of labelled steroids was usually greater than 80%.

Table I illustrates the use of the single solvent system. The column headed 'elution fraction' indicates the volume range within which the majority of each steroid can be recovered. The separations depend upon the respective polarity of the steroid[5].

Complete separation of progesterone from 17α- and 20α-hydroxyprogesterone can be achieved by increasing the column height to 38 cm. The resulting elution peaks are 12 ml for progesterone, and 16 ml for the other two progestins. The three classic

TABLE I

SEPARATION OF STEROIDS ON SEPHADEX LH-20 USING METHYLENE CHLORIDE AS THE SOLVENT SYSTEM

Column height, 25 cm; flow-rate, 25 ml/h.

Steroid	*Approx. elution peak (ml)*	*Elution fraction (ml)*
Corticoids		
11-Deoxycorticosterone	8	6–10
Corticosterone	13	11–16
Aldosterone	22	17–26
Cortisone	28	25–32
Cortisol	48	44–54
Other steroids		
Progesterone	8	6–10
17α-Hydroxyprogesterone	10	8–10
20α-Hydroxyprogesterone	10	8–12
Testosterone	11	10–13
Dihydrotestosterone	11	10–13
Oestrone	32	28–37
Oestradiol	85	70–90

oestrogens, oestrone, oestradiol, and oestriol, can be rapidly separated on a 6-cm column (the elution peaks are 8, 18 and 100 ml, respectively).

The system described in this communication is particularly applicable to steroid radioassay procedures. The use of a single solvent is advantageous in that column preparation is simplified and no solvent mixtures have to be equilibrated.

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