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Note

Convenient system for the simultaneous separation of 11-deoxycortisol and aldosterone by Sephadex LH-20 multiple column chromatography

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In routine separations of multiple adrenal steroids on Sephadex LH-20 columns [1], an overlapping zone of 10 to 30% was constantly found between the elution peaks of 11-deoxycortisol (S) and aldosterone (A) when using solvent systems and column dimensions which allow the simultaneous and automated isolation of a variety of both very unpolar (e.g., progesterone) and very polar (e.g., cortisol) steroids from the same plasma extract [2] prior to radioimmunoassay. Such an overlapping zone between the peaks of two tritiated steroids leads either to very low recoveries of both steroids if the overlap is eliminated or to erroneously high recoveries in one and erroneously low recoveries in the other steroid when no fraction between the two peaks is omitted. The use of differently labelled S and A was disadvantageous, too, because of the low specific activity of the ^{14}C-labelled compound whereby large amounts of steroid would have been introduced into the sensitive radioimmunoassay system.

A variety of organic solvent systems was therefore tested in combination with different LH-20 column dimensions in order to obtain a complete and convenient separation of S and A. The present communication reports a simple, rapid and highly practicable chromatographic system for the separation of these adrenal steroids on Sephadex LH-20 which to our knowledge has not yet been described to date.

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EXPERIMENTAL

Tritiated steroids, all with a specific radioactivity of 40—60 Ci/mmole, were purchased from New England Nuclear (Dreieich, G.F.R.) and were repurified every three months on Sephadex LH-20 using methylene chloride—methanol (98:2) as solvent. Sephadex LH-20 was obtained from Deutsche Pharmacia (Freiburg, G.F.R.). Analytical-reagent grade solvents (E. Merck, Darmstadt, G.F.R.) were used without further purification.

Sephadex LH-20 was allowed to swell overnight in the solvent system methylene chloride—acetone (80:20) and then poured into borosilicate-glass columns (44 × 1.1 cm I.D.) with a ground-in connector at the top and a frit (Schott, Mainz, G.F.R.) having 40—90-μm wide pores as gel support at the bottom. Below the frit, a PTFE stopcock was attached. With the stopcock permanently open, the gel was allowed to settle by gravity. A total of ten columns was packed at the same time; a gel height of 40 cm was reached in all columns after ca. 40 min. After packing, all columns were rinsed with one gel volume (38 ml) of the solvent and were left stoppered until use.

Radioactive steroids or the unseparated, dried S and A fractions from the initial chromatographic step [2] were applied to the almost dry top of the gel in two 250-μl portions of the solvent delivered by means of 1-ml tuberculin glass syringes. The columns were eluted by gravity flow with the solvent from a graduated, cylindrical (100 ml) reservoir which had been attached on top of the columns immediately after sample application [3]. Starting from the 60-ml mark, the eluate could be conveniently fractionated by volume as indicated by the actual solvent level in the reservoir. For routine separations, when ten columns were eluted together, the collection limits for S and A were appropriately marked on the calibration scale of each reservoir.

For localization studies, 1-ml fractions of the eluate were collected into miniature scintillation vials (16 × 54 mm; Zinsser, Frankfurt, G.F.R.) and were then counted with 5 ml of a toluene-based scintillation fluid in a Nuclear Chicago Isocap 300 scintillation counter (Searle, Heusenstamm, G.F.R.).

RESULTS AND DISCUSSION

With the solvent system methylene chloride—acetone (80:20), a complete separation of S and A from each other was obtained on 40-cm Sephadex LH-20 columns (Table I).

Resolution of the less-polar steroids tested was incomplete, whereas more-polar steroids like cortisone could not be eluted in a distinct peak. The 3-ml overlap zone between corticosterone and S did not create any problems since these two steroids were already completely separated from each other by a prior chromatographic step [2]. As the elution flow-rate averaged 40 ml/h, the separation of S and A could be completed within ca. 75 min.

In Fig. 1, the elution profiles of tritiated S and A from the ten parallel columns are shown. Both location and width of the steroid peaks show only limited variation. Nevertheless, the collection limits of each column were individually established by fraction-to-fraction analysis during the first elution. Due to a slight shrinkage of the gel column of ca. 2.5 cm after 14 elu-

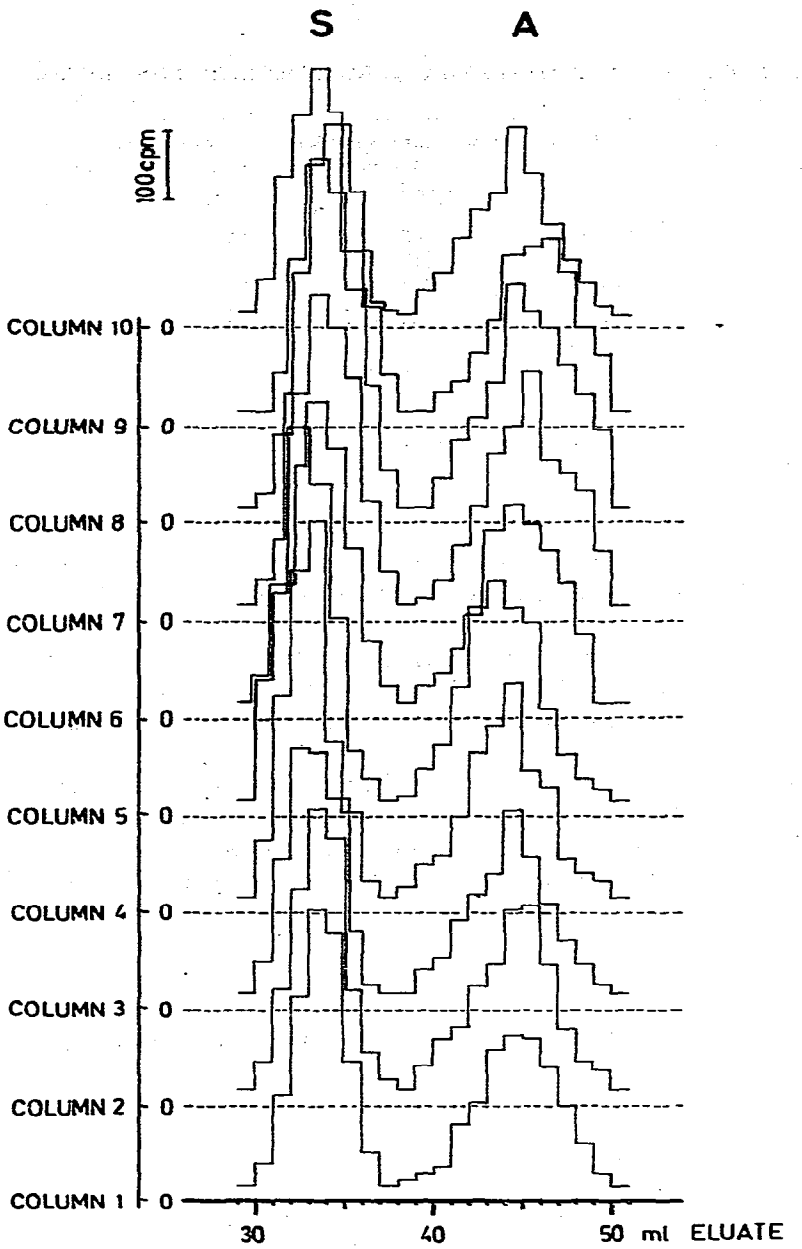

Fig. 1. Inter-column variation of the chromatograms of tritiated 11-deoxycortisol (S) and aldosterone (A) obtained at the 15th elution of ten LH-20 columns which had been packed at the same time.

tions, the elution volumes of S and A decreased by 2—4 ml, as can be seen by comparing the data in Table I with those in Fig. 1 which were obtained at the 15th elution of the columns. Because of this shifting, S and A were collected 2—4 ml earlier, as soon as the recoveries of tritiated S and A were

TABLE I

ELUTION VOLUMES AND COLLECTION LIMITS OF PLASMA STEROIDS ISOLATED ON FRESHLY PACKED 40-cm SEPHADEX LH-20 COLUMNS

Steroid	Elution volume (ml)	Collection limits* (ml)	Limits marked on reservoir*,** (ml)	Fraction pool volume collected* (ml)
Progesterone	19	(17—21)	(43—39)	(4)
11-Deoxycorticosterone	22	(19—24)	(41—36)	(5)
17-Hydroxyprogesterone	26	(23—28)	(37—32)	(5)
Testosterone	29	(27—31)	(33—29)	(4)
Corticosterone	32	(29—35)	(31—25)	(6)
11-Deoxycortisol	36	32—39	28—21	7
Aldosterone	48	44—52	16—8	8
Cortisone	>60	—	—	—

*Data for incompletely separated steroids are shown in parentheses
**Originating from a starting volume of 60 ml.

found to be slightly decreasing which usually occurred after 10—15 elutions. All columns were packed afresh after about 30 elutions using either fresh or re-purified [4] Sephadex LH-20.

Recoveries of tritiated S and A after extraction and two subsequent chromatographies were 61.4 ± 7.8% and 55.7 ± 7.7% (mean of 120 two-fold elutions ± S.D.), respectively, with mean coefficients of variation of 11.2 and 10.5% between ten parallel pairs of columns.

Among the ten different solvent systems tested, methylene chloride—acetone (80:20) provided by far the fastest, most reliable and economical separation of S and A. The consistently high steroid amounts recovered allowed sensitive radioimmunological quantification of these steroids in the same small plasma sample [5].

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