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SUMMARY

Reversed-phase partition chromatography of some neutral C_{27}-hydroxy and keto-steroids using aqueous isopropanol as moving phase and chloroform in heptane as stationary phase is described. The method has proved useful for separating cholesterol from its autoxidation products.

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Short Communications

Chromatography of follicle-stimulating hormone (FSH) on hydroxyl apatite

Calcium phosphate has been used by several groups of investigators[1,2] to purify crude urinary gonadotropins. Recently, TISELIUS *et al.*[3] have reported that hydroxyl apatite is an excellent material for the chromatography of proteins. Using column chromatography with this substance it has been possible to prepare FSH with a high specific activity.

Swine FSH prepared by the method of STEELMAN *et al.*[4] and having an activity of 2.5 times the Armour Standard (264-151X)[5] was first purified by the use of diethylaminoethyl (DEAE)-cellulose*. A 12 × 450 mm column of DEAE-cellulose (0.5 mequiv./g) was equilibrated at pH 7.0 with 0.005 *M* sodium phosphate buffer. The FSH (750 mg), dissolved in 40 ml of the same buffer, was placed on the column and followed with 40 ml buffer. A solution consisting of 80 ml 0.025 *M* NaCl in 0.005 *M* phosphate buffer, pH 7.0, was then used to remove inactive impurities. The FSH activity was then eluted with 120 ml of a solution consisting of 0.065 *M* NaCl in 0.005 *M* phosphate, pH 7.0. The yield after dialysis and lyophilization was approximately 150 mg with an activity of 8–10 times standard.

Hydroxyl apatite was prepared according to TISELIUS *et al.*[3]; however, it was found that a bulking agent was necessary to insure an adequate flow rate. Solka Floc (α-cellulose*) washed with dilute alkali and equilibrated with the 0.01 *M* phosphate buffer, pH 6.8, was found to be satisfactory. A mixture of approximately one part of hydroxyl apatite to one part of Solka Floc did not appreciably alter the chromatographic properties of the hydroxyl apatite with respect to FSH. 100 mg FSH, purified as above, was dissolved in 15 ml 0.01 *M* phosphate buffer, pH 6.8 and placed on a column (10 × 300 mm) of hydroxyl apatite equilibrated with 0.01 *M* phosphate, pH 6.8. The sample was followed successively with 0.01 *M*, 0.04 *M* and 0.2 *M* phosphate solutions at a constant pH of 6.8 using a flow rate of 10–15 ml/h. Fig. 1 shows the elution pattern obtained by collecting

* Obtainable from Brown Paper Co.

3.0 ml fractions using ultraviolet absorption at 280 mμ as an index of protein concentration. The total optical density is the observed optical density multiplied by the sample volume (3.0 ml). The major part of the FSH activity as determined by the method of STEELMAN AND POHLEY[5] was observed in the Fr II eluted with 0.04 *M* buffer. Prior experiments showed little or no optical density or activity was eluted with 0.02 *M* solutions. The activity of Fr II was 20–25 times standard. Rechromatography of the Fr II resulted in a product with an activity of 35–40 times standard, but the yield of activity was only 40–50%. It has been found that loss in activity of high-potency fractions is accompanied by an increase in Fr I and to a lesser extent Fr III with a concomitant decrease in Fr II, suggesting that the highly-active FSH is unstable. Fractions with identical activities, but prepared solely by DEAE-cellulose chromatography, have been shown to be relatively unstable in the lyophilized state.

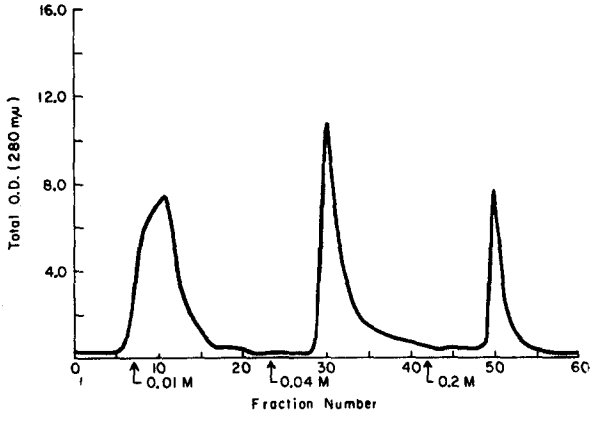

Fig. 1. The chromatography of swine pituitary FSH on hydroxyl apatite.

	% *O.D.*	*Activity*
Fr I (1–28)	48	< 8
Fr II (29–40)	36	20–25
Fr III (41–60)	16	< 8

Ultracentrifugal analysis of rechromatographed Fr II has been conducted at pH 8.6 in 0.05 ionic strength veronal buffer. Two components were observed, the major one constituting about 70% of the total and sedimenting at a rate approximately twice that of the minor component. The estimated sedimentation constant ($S_{20,w}$) for the major component was 2.0–3.0. Paper electrophoresis experiments using veronal buffer at pH 8.6 indicated that the product contained one major component with a small amount of a contaminant with a slightly lower mobility. The product of comparable activity obtained with DEAE-cellulose using non-enzymically-treated FSH exhibited the same ultracentrifugal and electrophoretic components and properties.

These findings are in contrast to those of STEELMAN *et al.*[6] with swine FSH which had been treated with pancreatin. The FSH thus prepared was apparently homogeneous by paper electrophoretic, ultracentrifugal and chromatographic analyses. The enzymically-treated product was 35–40 times standard and it has been found to retain its full activity in the lyophilized state for more than 15 months. A comparison of the stabilities of the two types of FSH preparations will be published later.

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Departments of Biochemistry, Baylor University College of Medicine, University of Texas, M. D. Anderson Hospital, Houston, Texas (U.S.A.) SANFORD L. STEELMAN

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