A Simple Method for Preparation of Highly Purified Vasopressin.* (23542)

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In the course of a series of experiments toward the characterization in the hypothalamus of the mediator of ACTH-release(1) it became imperative to study the possible role of vasopressin in this phenomenon. This required that milligram quantities of highly purified vasopressin be available for biological studies and also for physico-chemical comparison with the ACTH-releasing substance which, on the basis of the extraction procedure and other criteria, appeared to be related in some ways to the pressor principle(1). Highly purified arginine-vasopressin (AVP) and lysine-vasopressin (LVP) have been prepared by methods which all require days or weeks (2,3,4) for completion and usually large quantities of starting material. The method proposed here permits the separation of highly purified vasopressin in a few hours and with yields high enough to allow starting with reasonably small quantities of crude material. Successful chromatography of vasopressin has been described using Amberlite IRC-50 ion exchange resin with phosphate(5) or ammonium acetate(6) buffers. Condliffe has used partition chromatography(7). All these procedures suffer from the disadvantage of extremely low flow rates and capacity. In the studies described here, high capacity and flow rate have been obtained using a cellulose ion exchanger.

Methods. A carboxymethyl cellulose ion exchanger prepared according to the method of Peterson and Sober(8) and possessing a capacity of 0.65 meq/g was used for the present studies. Starting material for the chromatography was obtained by fractionation of Proto-pituitrin (Parke-Davis) by the method of Kamm *et al.*(9). For the LVP a fraction with a potency of 22 pressor units/mg (equivalent to combined fractions "e" and "f" in the original Kamm procedure) was used, while a similar fraction from beef Proto-pituitrin having a potency of 35 u/mg was used for the AVP. Chromatography was carried out in glass tubes approximately 1 cm in diameter and with a height of 25 cm or 60 cm. Material to be chromatographed was dissolved in 0.02 M ammonium acetate buffer, pH 6.0, and applied to the column previously equilibrated with this same buffer. Development of the column was then carried out using a gradient through a 250 ml mixing chamber(10) to either a 0.1 M or a 0.2 M ammonium acetate buffer, pH 7.0. Protein was measured by U.V. absorption at 275 mμ and the Folin-Lowry reaction(11). After the vasopressin peak was located by bioassay of aliquots of the effluent the appropriate fractions were pooled and lyophylized. Using an all-glass lyophilizer and a vacuum pump operating at 0.1 mm of Hg, all detectable ammonium acetate was removed after the material was redissolved in water and lyophilized one more time at temperatures not exceeding 25°C. Material dried in this manner was then used for the final determination of specific activity. Assays for pressor activity were carried out using the rat blood pressure method of Dekanski(12). For determinations of the activity contained in aliquots of the effluents, bracketed assays were used. To measure the specific activity of the final lyophilized products, four-point-assays in duplicate were run and calculated according to Bliss(13). All assays were done using USP posterior pituitary Reference Standard with a stated potency of 0.4 u/mg (recently revised downward from 0.47 u/mg).

Results. Fig. 1 shows the chromatogram of 750 mg of partially purified lysine vasopressin (22 u/mg) on a 25 cm colum containing 2.5 g of exchanger, with hold-up volume of 18 ml and operated at a flow rate of

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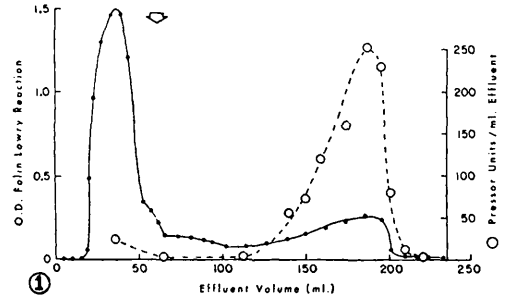

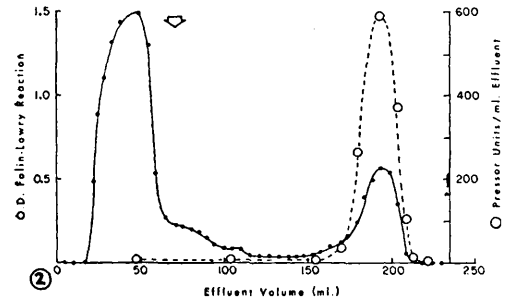

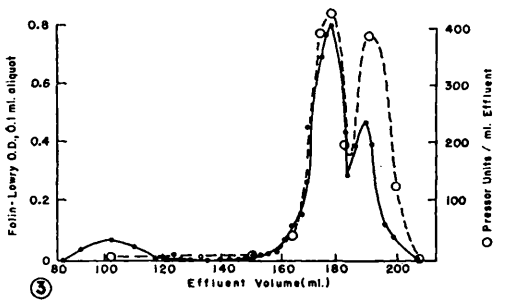

FIG. 1. Chromatography of lysine vasopressin on carboxymethyl cellulose. 750 mg with potency of 22 u/mg applied to the column (1 × 25 cm) in 0.02 M ammonium acetate buffer, pH 6. Gradient to 0.1 M ammonium acetate buffer, pH 7, through 250 ml mixing chamber was begun at the point marked by the arrow. ●—Folin-Lowry color, 0.05 ml aliquots. ○—Pressor activity of effluent.

FIG. 2. Chromatography of arginine vasopressin on carboxymethyl cellulose. 500 mg with a potency of 35 u/mg applied to the column (1 × 25 cm) in 0.02 M ammonium acetate buffer, pH 6. Gradient to 0.2 M ammonium acetate buffer, pH 7, through 250 ml mixing chamber was begun at the point marked by the arrow. ●—Folin-Lowry color, 0.1 ml aliquots. ○—Pressor activity of effluent.

FIG. 3. Chromatography of arginine and lysine vasopressin mixture on carboxymethyl cellulose. 15 mg lysine vasopressin (250 u/mg) and 10 mg arginine vasopressin applied to the column (1 × 60 cm) in 10 ml pH 6, 0.02 M ammonium acetate buffer. Gradient to pH 7, 0.2 M ammonium acetate buffer through 250 ml mixing chamber started after 12 ml of effluent. Small peak at 100 ml effluent vol represents an impurity present in this particular lysine vasopressin sample. ●—Folin-Lowry color, 0.1 ml aliquot. ○—Pressor activity of effluent.

approximately 30 ml per hour. 60 mg of vasopressin were isolated from the pressor area (150-205 ml effluent) with a specific activity of 260 u/mg. Hydrolysis of this material with 6N HCl showed all the amino acids of LVP to be present(3,14) plus traces of alanine and histidine as revealed by paper chromatography of 1 mg of the hydrolyzate.

Rechromatography of this material on a longer column gave a product with essentially the same specific activity, but containing only the amino acids of LVP. The potency and purity of this material thus appear as good as that reported for LVP obtained by other methods(3).

Fig. 2 shows a similar chromatogram of partially purified AVP (500 mg, 35 u/mg) as with the LVP, except for the elution gradient which was to a 0.2 M ammonium acetate buffer, pH 7.0. The pressor material (172-212 ml effluent yielded 40 mg after lyophilization) assayed 380 u/mg and contained only the 8 amino acids of AVP(2). This material thus has a lower potency than the most potent material obtained by Popenoe *et al.*(15), although subsequent preparations by the Cornell group have exhibited lower potencies, yet contained only the 8 amino acids of AVP(7, 16). Thus we feel that this represents a reasonably good purification of AVP.

From the difference in the elution gradient employed for LVP (Fig. 1) and AVP (Fig. 2) it was anticipated that the two hormones could be resolved by chromatography on carboxymethyl cellulose. Accordingly, 15 mg of LVP (250 u/mg) and 10 mg of AVP (380 u/mg) were mixed and applied to a column 1 x 60 cm with 40 ml hold-up volume and 5 g exchanger in pH 6.0, 0.02 M ammonium acetate buffer and a gradient to 0.2 M ammonium acetate buffer pH 7.0 used to develop the column. Under these conditions it can be seen (Fig. 3) that LVP and AVP are resolved. Only the second pressor peak gave a positive Sakaguchi reaction. Thus the AVP showed a greater affinity for the column, as would be expected from its higher isoelectric point, 10.9(5,17), compared to 10.0 for LVP.† The higher ratio of activity to Folin-Lowry O.D. for the AVP peak in Fig. 3, reflects the greater

† Unpublished results, D. N. Ward.

potency per unit weight for AVP(3,15).

Summary. Both arginine and lysine vasopressin have been obtained in a high degree of purity by means of chromatography on carboxymethyl cellulose using ammonium acetate as a volatile buffer and differential elution gradients. Conditions for the resolution of LVP and AVP by chromatography on this same exchanger are described. By combining efficient recovery of activity, high flow rates for the chromatography, and rapid removal of buffers by lyophilization, a simple means of purifying vasopressin is proposed.

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Effect of a Histamine Releaser (48/80) upon Development of Walker Tumor.* (23543)

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The possibility of depleting a tissue of its histamine content by repeated injection of compound 48/80(1) suggests a method by which to evaluate the role of histamine in tissue injury. This method was first used in our studies on inflammation. In rats treated with compound 48/80 or various histamine releasers the tissue responsiveness is either enhanced or decreased, depending upon the nature of the irritant utilized(2).

In the course of our studies on the relationship between inflammation and cancer we thought it would be interesting to investigate the influence of compound 48/80 upon development of a transplanted tumor. The present experiments suggest that this histamine releaser, especially when given as pretreatment, is particularly effective in enhancing the development rate of Walker tumor.

Materials and methods. Three experimental series were made with female Sprague-Dawley rats of an average initial body-weight of 60 g (range 58 to 64 g). It was planned to determine whether the development of the tumor would be influenced by 48/80‡ administered before transplantation (Exp. 1), during tumor-growth (Exp. 2), and after the tumor is well developed (Exp. 3). In each experiment the compound was injected subcutaneously, twice daily, for 8 days; doses

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