Ion-exchange Chromatography of Oxytocin, Arginine-vasopressin, and Lysine-vasopressin.* (20838)

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In the course of the isolation of hormones from beef and hog posterior pituitary glands, it was found that oxytocin prepared from beef sources could not be differentiated from that obtained from hog by the technics employed (1). Vasopressin, however, had a different composition in these two species. The vasopressin prepared from beef glands was differentiated from that found in hogs in that the former contained arginine; this material has been termed *arginine-vasopressin*. Hog glands have yielded a vasopressin characterized by the presence of lysine in place of the arginine found in vasopressin from beef sources, and this has been termed *lysine-vasopressin*(2,3).

Previous attempts in this laboratory to separate and define these preparations as chemical entities have involved principally the use of countercurrent distribution technics (2,4) and zone electrophoresis on various supporting media(5,6). Filter paper electrophoresis has proved to be an extremely useful analytical tool for the routine detection of oxytocin; however, the method does not offer a critical separation of lysine- and arginine-vasopressin since the isoelectric point of lysine-vasopressin is only slightly lower than that of arginine-vasopressin, and their pH-electrophoretic mobility curves are similar(7). Hence there is a need for another method of separating the 2 known vasopressins on an analytical scale. For this purpose, an investigation has been made of the chromatographic behavior of these 3 hormones on the carboxylic acid ion exchange resin Amberlite IRC-50 (XE-64). This resin has been found to be suitable for the chromatography of cytochrome c(8), pancreatic ribonuclease(9), egg lysozyme(10), and spleen lysozyme(11).

Methods. Chromatography was carried out on 0.9 x 30 cm columns of ion exchange resin Amberlite IRC-50 (XE-64). The preparation of the resin and operation of the column was carried out according to the procedures of Hirs, Moore, and Stein(9) and Tallan and Stein(10). The rate of flow of solvent through the column was 1.5 ml per hour. 0.5 ml fractions of the effluent were collected for analysis. Sodium phosphate buffer was used as the eluting agent; the buffer was saturated with chloretone as a preservative. Analysis of effluent fractions was carried out with the modified Folin reagent(12). These fractions were collected in 13 x 100 mm pyrex test tubes; 3 ml of reagent C were added followed by the addition of 0.3 ml of dilute Folin reagent. Readings were made at 750 mμ in the Beckman spectrophotometer. Oxytocic activity was determined by the method of Coon (13) and pressor activity was measured on cats anesthetized with sodium phenobarbital. *Oxytocin* in these studies was prepared from a crude material of 50 units per mg supplied by Armour and Co. The material was extracted with *sec*-butyl alcohol-acetic acid; the butyl alcohol phase was then washed with phosphate buffer(14). After countercurrent distribution in *sec*-butyl alcohol-acetic acid this material had a potency of approximately 500 oxytocic units per mg. Arginine-vasopressin was obtained from a product extracted from beef glands which had an activity of 50 pressor units per mg. This product was subjected to electrophoresis, using procedures previously described(6), on a cellulose‡ supporting medium(7). A pyridine-acetate solu-

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‡ Solka-Floc, SW-40-A, Brown Co., New York City.

tion at pH 5.7, $\Gamma/2 = 0.1$, was employed as a buffer. The active fractions were collected, filtered, and lyophilized to dryness; the arginine-vasopressin so obtained had a potency of 400 pressor units per mg dry weight. Lysine-vasopressin was similarly prepared from a starting material with a potency of 50 pressor units per mg that was obtained from hog sources; the final product had a potency of 210 pressor units per mg.

Results. Preliminary equilibration experiments(9) were performed in a search for a suitable phosphate buffer concentration and pH level for chromatography of arginine-vasopressin; at pH 6.95 in 0.2 M sodium phosphate buffer sufficient absorption of the vasopressin occurred to insure success of a column. When arginine-vasopressin was placed on the IRC-50 column with this buffer as the eluting agent, a single peak emerged from the column at approximately 70 effluent ml. Assays for pressor activity showed a reasonable but not perfect correspondence with the Folin phenol color, indicating the presence of some inactive impurities. Lysine-vasopressin was chromatographed in a similar manner at pH 6.95 in 0.2 M phosphate buffer; it emerged at 40 effluent ml in a peak that contained the expected pressor activity. Oxytocin, being less basic than lysine-vasopressin, would be expected to precede lysine-vasopressin on elution from the column. Accordingly, 0.6 mg of oxytocin, 0.6 mg of lysine-vasopressin, and 0.7 mg of arginine-vasopressin were dissolved in 0.5 ml of the buffer and chromatographed in the usual manner. The analysis of effluent

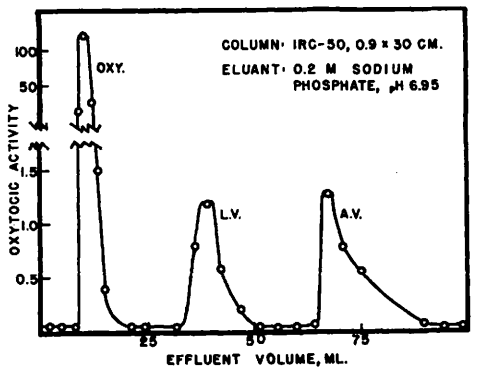

FIG. 1. Chromatography of a mixture of oxytocin (Oxy.), lysine-vasopressin (L.V.) and arginine-vasopressin (A.V.).

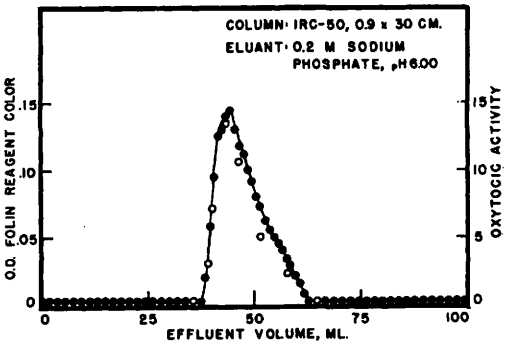

FIG. 2. Chromatography of 0.8 mg of oxytocin. ●, Folin reagent color; ○, oxytocic activity.

fractions for their oxytocic activity is shown in Fig. 1. Oxytocin emerged after the first column volume without any retardation. Lysine-vasopressin emerged at 40 ml, while arginine-vasopressin was retarded to the extent that its peak appeared at 70 ml. While this experiment demonstrated a separation of the three known posterior pituitary hormones, it does not represent a true chromatography of oxytocin since this peptide emerged as a narrow peak after the first column volume.

Chromatography of oxytocin was accomplished on IRC-50 by employing 0.2 M sodium phosphate buffer at pH 6.00. Under these experimental conditions oxytocin appeared with a peak at 45 effluent ml, as shown in Fig. 2. Oxytocic assays performed on several fractions showed a close correspondence between oxytocic activity and phenol color. *Synthetic oxytocin*§(15) was found to appear in the same position on the chromatogram and the shape of the curve was similar to that of Fig. 2. When a mixture of 0.37 mg of natural oxytocin and 0.34 mg of synthetic oxytocin was chromatographed under the same conditions the results were again essentially the same as those of Fig. 2.

Discussion. IRC-50 resin appears to possess a high degree of resolution in that it can separate two highly basic peptides of similar structure(16,17), lysine-vasopressin and arginine-

§ The *synthetic oxytocin* is a sample of the synthetic octapeptide amide with the hormonal activity of oxytocin(15). From the many comparisons of the synthetic material and natural oxytocin which have been made there appears no doubt that the synthetic product represents oxytocin.

vasopressin. Both of these hormones have been shown to possess inherent oxytocic activity(18,19). It is therefore possible to demonstrate the presence of the vasopressins by means of an assay for oxytocic activity. The fowl depressor assay of Coon(13) was used as a measure of the oxytocic activity in the present work. This method has previously been shown to give an estimate of oxytocic activity which is high by a factor of 2.7 when there is present predominant amounts of pressor activity(18). The results presented in Fig. 1 indicate that all three of the known hormone preparations derived from the posterior pituitary glands are potent oxytocic agents.

The chromatography of oxytocin required the resin column to be buffered at pH 6.00. By comparison, Hirs, Moore, and Stein reported that ribonuclease can be chromatographed at pH 6.47. Since oxytocin and ribonuclease have nearly similar isoelectric points at pH 7.7 and 7.8 respectively, the rather low pH required for the chromatography of oxytocin is probably due to the fact that oxytocin is virtually uncharged at its isoelectric point(6). Thus a sufficient degree of ionization of the molecule to enable it to react with IRC-50 is not achieved until the pH is lowered to 6.00. The oxytocin preparation examined in Fig. 2 appears to consist of a single active component in which the oxytocic activity corresponds to the phenol color. Investigation of the behavior of synthetic oxytocin on IRC-50 has shown that it occupies the same position in the chromatogram as the natural material, and that a mixture of synthetic and natural oxytocin chromatographs as a single substance.

Summary. Oxytocin, lysine-vasopressin, and arginine-vasopressin can be chromatographed on ion exchange resin IRC-50 (XE-64). Complete separation of the three hormones was achieved by passage through a column buffered at pH 6.95 with 0.2 M phosphate buffer; under these conditions lysine-vasopressin and arginine-vasopressin emerged at 40 and 70 effluent ml respectively, while oxytocin passed through without retardation. At pH 6.00, with 0.2 M phosphate buffer as the eluting agent, oxytocin, synthetic oxytocin, and a mixture of natural and synthetic oxytocin chromatographed as a single substance.

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