CHROM. 3382

Purification of antidiuretic hormone by high voltage electrophoresis*

During the course of a project involving antidiuretic hormone, it was realized that milligram quantities of this substance would be required. Commercial sources indicated that antidiuretic hormone from animals was electrophoretically, and therefore chemically, impure. Further investigation revealed that the hormone is most difficult to prepare in large quantity by a synthetic route[1]. Hence, it seemed most logical to attempt to purify the commercially available product.

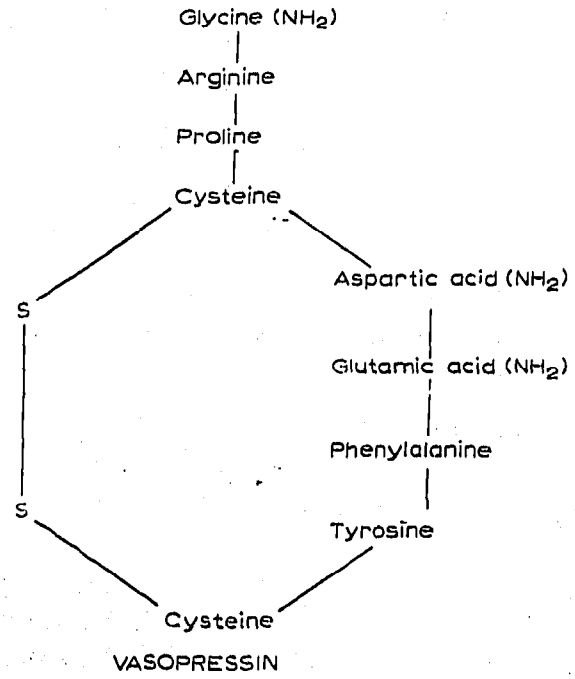

Fig. 1. Structure of arginine-8-vasopressin (human antidiuretic hormone).

Human antidiuretic hormone is arginine-8-vasopressin (Fig. 1)[2]. If arginine is replaced by leucine, and phenylalanine by isoleucine, the structure becomes oxytocin, a hormone with uterus-contracting and milk-ejecting properties. Oxytocin is the most physiologically active contaminant of vasopressin preparations from animal sources; both vasopressin and oxytocin are stored in the posterior lobe of the pituitary gland, the most common commercial source of these hormones[3]. As early as 1941, duVigneaud and associates suggested that these two compounds could be separated by electrophoresis[4]; later work showed that they have sufficiently dissimilar isoelectric points (pH 10.9 for vasopressin and pH 7.7 for oxytocin[5]) for good separation by this technique. At a pH of 9.4, then, vasopressin would migrate toward the cathode and oxytocin toward the anode. Accordingly, preparative electrophoresis seemed to be a feasible method for large-scale preparation and purification of antidiuretic hormone.

High-voltage electrophoresis was chosen over the orthovoltage technique since

* The mentioning of trade names and manufacturers does not constitute approval or official endorsement of these products by the U.S. Government.

the high-voltage procedure will effect a separation in a much shorter period of time. Time can be of great importance in manipulating polypeptides, since these compounds are readily attacked by omnipresent bacteria and can be thermally inactivated relatively easily under conditions of electrophoresis.

The instrument selected for the procedure was manufactured by Savant Instruments Incorporated. The electrophoresis chamber contained 6 l of buffer in each of two compartments, overlaid with 50 l of high flash point paint thinner (Varsol B®) as a coolant. Supplemental cooling was accomplished by tap water circulating through coils immersed in the paint thinner. The buffer employed in the procedure consists of a 0.05 *M* solution of sodium tetraborate, adjusted to a pH of 9.4 with sodium hydroxide. The buffer was used for 10 to 12 electrophoretic runs before a change in pH was noted.

The starting material for the purification of vasopressin was a product manufactured by Parke-Davis, Pitressin®, an aqueous solution of the pressor and antidiuretic principle of the posterior pituitary gland. Forty milliliters of this solution was freeze-dried to reduce its volume for spotting on the filter paper. The resulting powder was taken up in 0.8 ml of distilled deionized water, a sufficient amount to just dissolve the solid material. The solution was applied as a streak with a disposable capillary pipet to the center of an 18 × 42 in. piece of filter paper. The width of the streak was kept to less than 0.5 cm, and was dried between applications with a forced-air drier. The paper was transferred to the support rack, carefully saturated with buffer, and immersed in the electrophoresis chamber. Electrophoresis was performed for 2 h at 2500 V. At the completion of the run, the rack was removed and set in front of a fan for air drying at room temperature. A strip was cut from each side of the paper, including about 0.5 cm of the streak. These strips were stained by dipping into ninhydrin solution and heated to 100°C for 5 min to develop the color. They were then returned to their original positions alongside of the unstained portion and fastened to it with tape. Several bands were noted on the stained portion (Fig. 2), corresponding to electrophoretically separable polypeptides. The band closest to the anodal side of the line of application migrated with an oxytocin standard. The compound of interest, vasopressin, corresponded to the band closest to the cathode. Fig. 3 shows an electrophoretogram of the purified product and synthetic arginine-8-vasopressin (furnished gratis by Sandoz Pharmaceuticals Inc.).

A narrow strip, corresponding to this latter band, was cut from the unstained portion of the sheet parallel to the line of application. This strip was placed in a glass chromatography column and eluted by slowly dripping deionized distilled water on the top of the strip. Twenty milliliters of eluate was collected over a period of about 15 min. This solution was freeze-dried, yielding 15 mg of a fluffy white powder, of which approximately 75 % was vasopressin and 25 % was sodium tetraborate from the buffer.

It was quite apparent that a considerable fraction of the starting material was not vasopressin. Some of it had the electrophoretic properties of oxytocin, while the remainder of the material migrating to the anode apparently consisted of lower molecular weight fragments.

Although the procedure described here is relatively simple and straight-forward, certain precautions should be observed for optimal results. It is important that Pitressin® solution be kept refrigerated and sterile prior to use in order to prevent

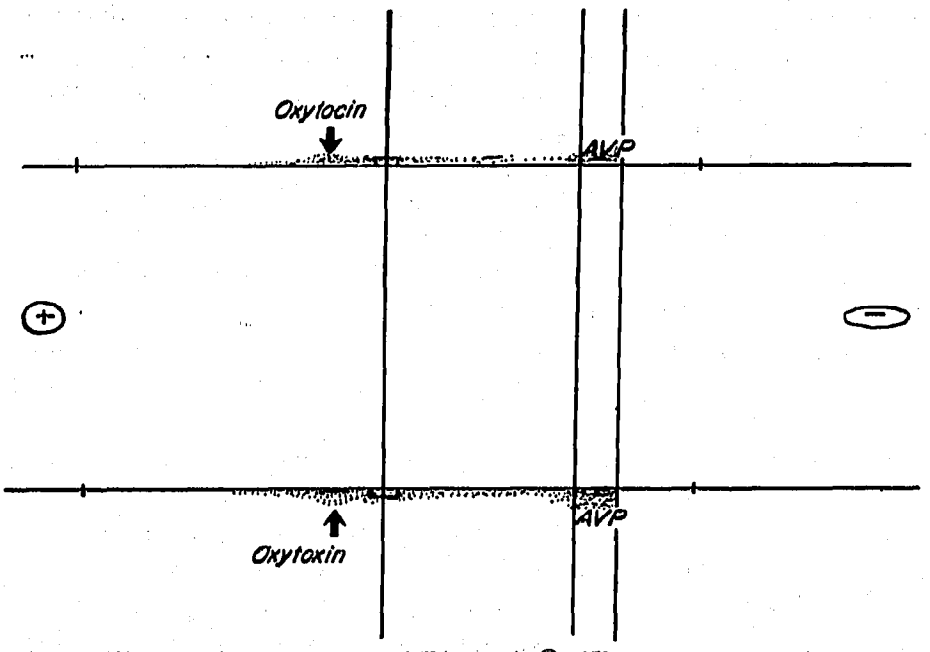

Fig. 2. Electrophoretogram of Pitressin®. The two outer edges are stained with ninhydrin, showing electrophoretic nonhomogeneity of the preparation. The parallel lines at the negative side of the center delineate the vasopressin fraction which is subsequently eluted. Arrows point to the fraction corresponding to oxytocin.

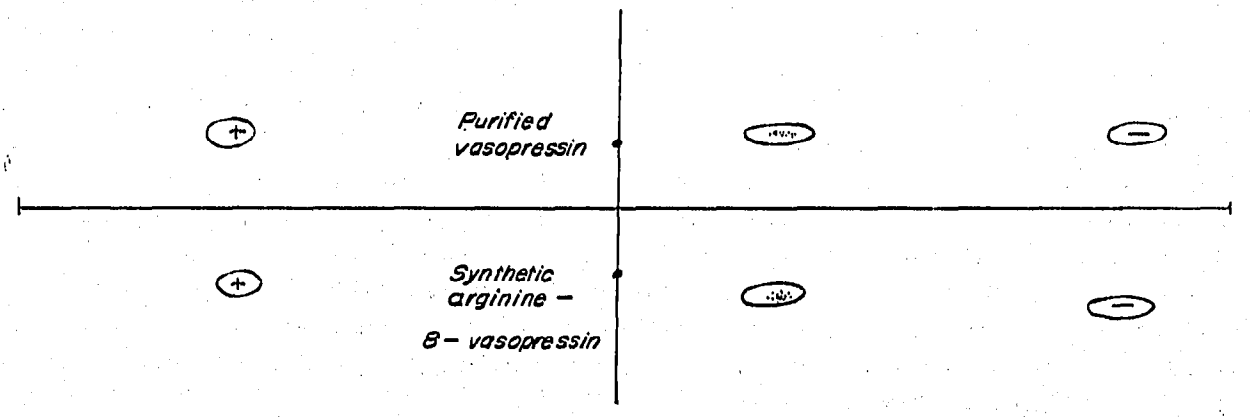

Fig. 3. Electrophoretogram of purified vasopressin and synthetic arginine-8-vasopressin, stained with ninhydrin. The mobilities of the two compounds are identical.

decomposition. Freeze-dried Pitressin®, in our experience, can be kept at freezer temperature without sterile precautions for several months. When it is reconstituted with water, it should be electrophoresed promptly. The temperature of the electrophoresis chamber should be kept at room temperature or only slightly above during the run. Electrophoresis current should be kept below 200 mA so that suspension of water in the coolant is minimal, thereby reducing markedly elution of the Pitressin® from the paper. Buffer should be applied to the paper very carefully; it should be allowed to wet the streak by osmotic action rather than by direct application, which causes the applied material to assume a larger area on the paper. The band containing vasopressin should be eluted as soon as possible after drying the paper, but storing the strip overnight in a freezer does not seem to be detrimental. The eluate should be

freeze-dried immediately after elution; the resulting powder can be kept at freezer temperature for several months.

Purified antidiuretic hormone (vasopressin) can thus easily be obtained from commercial posterior pituitary extract by the use of preparative high voltage electrophoresis. The resulting product appears to be chemically identical to the synthetic hormone.

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Electrophoretic behaviour of copper(II) histidinate in the presence of the other copper(II) amino acid chelates

Recently[1] we studied the electrophoretic behaviour of copper(II) amino acid chelates at different pH values. We noticed that complexes of dicarboxylic amino acids and histidine behaved somewhat unusually. Splitting of the former into two distinctive Cu^{2+} positive spots may be due to the structure of these complexes, as suggested by PFEIFFER AND WERNER[2]. On the other hand at pH 11.98 copper(II) histidinate yields two spots, one of which moves to the anode and the second to the cathode. WIELAND AND FISCHER[3], in 1948, also observed the somewhat peculiar behaviour of copper(II) histidinate in the presence of copper(II) lysinate, *viz.* the formation of a third spot during the electrophoretic separation of the two complexes. They suggested that this third spot was the mixed complex copper(II) histidino–lysinate. Owing to the fact that coordination of histidine with Cu^{2+} ions is unusual (see for example references 4, 6, 8) and because the kinetic lability of copper(II) amino acid complexes in aqueous solution restricts the formation of mixed amino acid complexes, the authors have studied the electrophoretic behaviour of copper(II) histidinate in the presence of the other chelates more extensively.

Thus we found that the existence of the third spot described by WIELAND AND FISCHER is observable only in the case of copper complexes of basic amino acids (with the exception of α,β-diaminopropionic and α,γ-diaminobutyric acids owing