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Immunoassay of Plasma Vasopressin in Man

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Abstract. A radioimmunoassay for arginine vasopressin has been developed that is sensitive to the hormone at 1 pg/ml. When plasma was filtered on G-25 Sephadex, immunoreactivity was detected in three regions. Endogenous arginine vasopressin was the third and smallest of the peaks and was recovered just after the salt. By combining gel filtration with immunoassay, we have made precise measurements of arginine vasopressin in a few milliliters of plasma.

Introduction. Arginine vasopressin (AVP), a nonapeptide of the posterior pituitary gland, is the major hormonal regulator of water conservation in man and most other mammals. Many details of vasopressin physiology have been obscure because simple precise methods for measuring the hormone concentration in plasma have not been available.

We have developed a radioimmunoassay that is sensitive to the hormone at 1 pg/ml and in which AVP reacts much more strongly than lysine vasopressin or oxytocin, two other nonapeptide hormones that differ from arginine vasopressin by only one and two amino acids, respectively. However, when dilute whole plasma was assayed, the apparent concentrations of arginine vasopressin were several hundredfold higher than anticipated, failed to fluctuate appropriately in response to physiological manipulation or in disease states, and were not reduced substantially by dialysis of the plasma under conditions that completely removed vasopressin.

When purified arginine vasopressin was filtered on G-25 Sephadex in the presence of salt, the hormone adsorbed loosely to the gel and was recovered as a discrete peak beyond the salt. When plasma was filtered under these conditions, two peaks of immunoreactivity were recovered before the salt, and a third peak, representing endogenous hormone, was recovered as a peak beyond the salt in the same location as pure arginine vasopressin. By combining gel filtration and radioimmunoassay, we have obtained precise and reliable measurements of arginine vasopressin in a few milliliters of plasma.

Methods. Normal healthy adults and patients with polyuria were inpatients on a metabolic ward. Tobacco and medications were prohibited during the studies. After 15–30 min of recumbency, venous blood, 150–450 ml, was drawn into iced heparin-rinsed containers and centrifuged immediately at 4°C. The osmolality of plasma and of urine was measured by freezing point depression. A 50-ml aliquot of plasma was immediately mixed with 2 M ammonium acetate to give a final concentration of 0.03 M and centrifuged. The supernatant was applied to a 4.4 × 46 cm column of Sephadex

G-25 fine that had been equilibrated in 0.03 M ammonium acetate (pH unadjusted was 6.7), and 10-ml effluent fractions were collected. 2 ml of each fraction were transferred to a smaller tube and lyophilized for assay.

The antiserum for the assay, prepared in rabbits by repeated injections of commercial vasopressin (Pitressin, Parke-Davis) emulsified in Freund's adjuvant, was a gift of Dr. W. Wu.[1] Another antiserum, which was added in excess to measure the immunological reactivity of AVP-^{125}I, was prepared in rabbits by injection of pure AVP in adjuvant.[2] Coupling agents were not used to produce either serum. Sheep antibody to rabbit gamma globulin (fraction II Pentex) was a gift of Dr. S. W. Rosen.

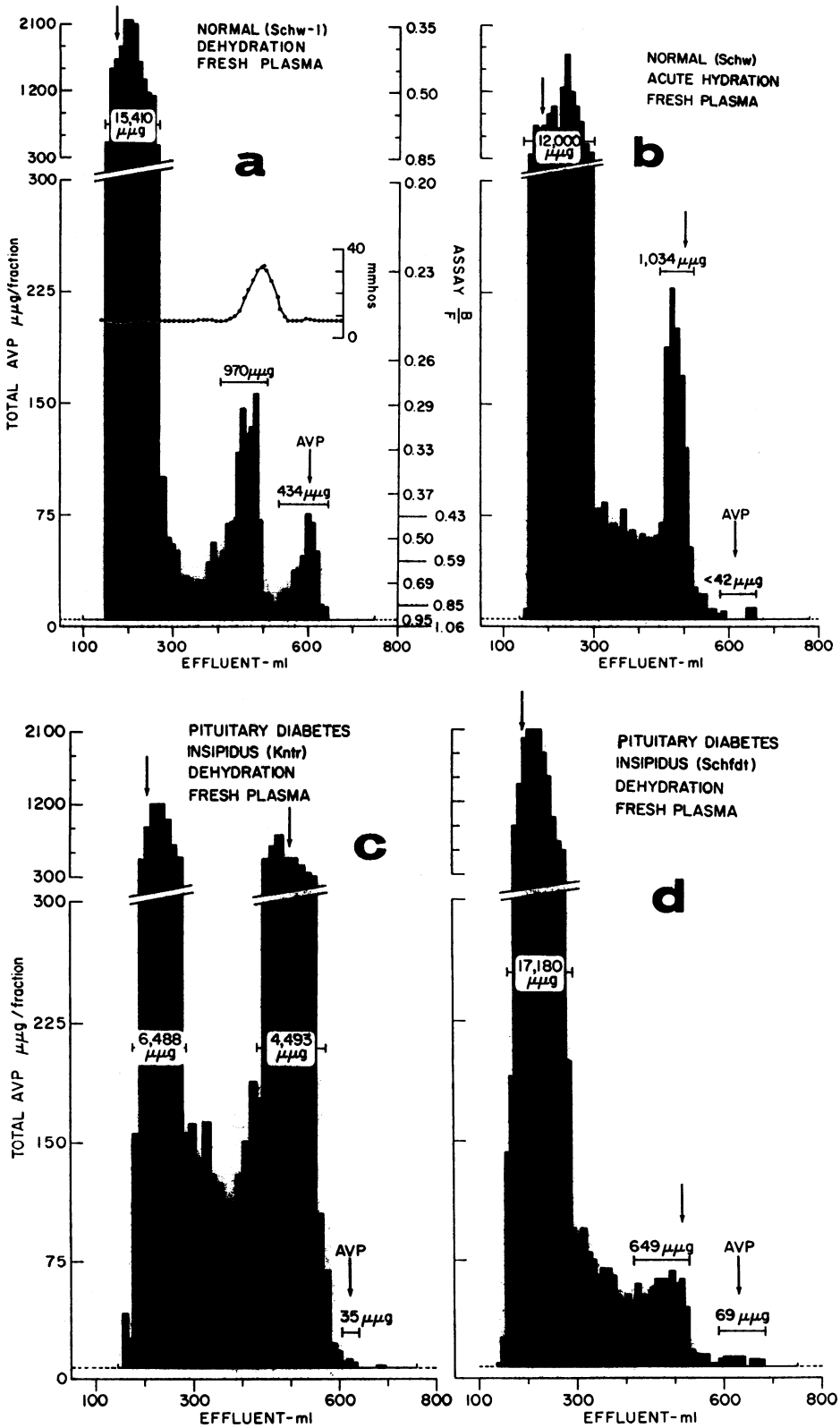
Arginine vasopressin, prepared from bovine glands,[3] yielded a single ninhydrin-positive spot on ascending paper chromatography in butanol:acetic acid:water, 4:1:5. Material from this region of the chromatogram, when eluted and hydrolyzed, yielded equimolar quantities of the appropriate amino acids. The AVP had a potency of 400–500 U/mg when assayed in toads *in vitro*[4] and *in vivo*[5] and in rats.[6]

AVP was reacted with ^{125}I (NaI carrier-free, Union Carbide) and chloramine T[7] but without metabisulfite[2] to yield mono-iodo AVP-^{125}I at 50 μCi/μg. AVP-I which adsorbs loosely to Sephadex G-25 was separated from iodide and unlabeled AVP by gel filtration in 0.2 M acetic acid,[2] yielding AVP-^{125}I at 1500 μCi/μg. The AVP-^{125}I used for the assay yielded a single radioactive spot on paper chromatography; with an excess of rabbit anti-AVP about 90% of it was precipitated by sheep anti-rabbit gamma globulin.

Diluent for the assay was sodium phosphate buffer 0.02 M pH 7.4 which contained NaCl, 0.14 M; crystalline bovine serum albumin (Pentex), 2.5 mg/ml; and normal rabbit serum (Pentex), 5 μl/ml. Each tube in the assay, in a total volume of 1 ml, contained AVP-^{125}I (1.0 pg), AVP (0–250 pg) or lyophilized fraction of plasma, and antiserum at a final dilution of 1:150,000. After 3 days, antibody-bound AVP-^{125}I was precipitated by the addition of anti-rabbit gamma globulin; the supernatants and precipitates were counted in an automatic well-counter. AVP at 1 pg/ml was detected routinely. Lysine vasopressin and oxytocin were more than 10 times and 1000 times less reactive.[8] The gel filtrations and immunoassays were performed at 4°C.

Results. When plasma from a dehydrated normal subject was filtered on Sephadex G-25, immunoreactivity was recovered in three regions (Fig. 1*a*). The largest peak (I) was in the void volume with the plasma proteins. The second peak (II), intermediate in size, appeared just before the salt. The third (III) and smallest peak eluted after the salt in a position that corresponded exactly to that of pure arginine vasopressin. Following hydration, peak III in plasma was virtually absent (Fig. 1*b*). Likewise in patients with pituitary diabetes insipidus (in whom polyuria is due to the lack of endogenous hormone) peak III of plasma immunoreactivity was absent even during severe dehydration (Fig. 1*c* and *d*). In patients with nephrogenic diabetes insipidus (in whom polyuria is due to congenital renal insensitivity to vasopressin) peak III of plasma immunoreactivity varied normally with the state of hydration (Fig. 1*e* and *f*).

In all plasmas from three normal adults and from three adults with nephrogenic diabetes insipidus the concentration of peak III immunoreactivity was closely related to the plasma osmolality, being very low at osmolalities below 285 mOsm/kg and rising progressively as osmolality increased above 285 (Fig. 2). The relationship between plasma osmolality and plasma vasopressin appeared to be the same in the normal adults as in the patients with nephrogenic diabetes insipidus, which suggested that life-long dehydration and hyperosmolality characteristic of these patients had failed to alter the sensitivity of their osmoreceptor-vasopressin mechanism. In both patients with pituitary diabetes insipidus.


NORMAL (Schw-1)
DEHYDRATION
FRESH PLASMA
a
15,410 μμg
970μμg
AVP
434μμg
40
mmhos
0
TOTAL AVP μμg/fraction
ASSAY B/F
EFFLUENT-ml
NORMAL (Schw)
ACUTE HYDRATION
FRESH PLASMA
b
12,000 μμg
1,034μμg
AVP
<42μμg
PITUITARY DIABETES
INSIPIDUS (Kntr)
DEHYDRATION
FRESH PLASMA
c
6,488 μμg
4,493 μμg
AVP
35μμg
PITUITARY DIABETES
INSIPIDUS (Schfdt)
DEHYDRATION
FRESH PLASMA
d
17,180 μμg
649μμg
AVP
69μμg

(*a*) Plasma (50 ml) from a normal male adult (Schw), obtained after 16 hr of complete fluid deprivation (plasma = 298 mOsm/kg; urine = 1138 mOsm/kg), was filtered on Sephadex G-25. An aliquot of each effluent fraction was lyophilized for assay. The total immunoreactivity in each fraction, expressed as picograms of pure arginine vasopressin (AVP), is shown on the vertical axis at the left; the actual values for bound/free of AVP-^{125}I in the assay (*B/F*) are shown on the vertical axis at the right. The additional *B/F* scale at the top is for the fractions in the void volume that were assayed at a further dilution. The minimum amount detected is indicated by the horizontal line at the bottom. The arrow at the left denotes the peak of A_{280}, and the arrow at the right indicates the characteristic location of pure AVP. The location of the plasma salt was determined by measurement of electroconductivity (*circles*) and is indicated in subsequent figures by an arrow. The total immunoreactivity in each of the zones is given over the horizontal brackets.

(*b*) 50 ml of plasma from a normal male adult (Schw) obtained after he had ingested 1000 ml of water over a 90-min period. Plasma = 284 mOsm/kg; urine = 79 mOsm/kg.

(*c*) 40 ml of plasma from a male with pituitary diabetes insipidus (Kntr) obtained after 5 hr of complete fluid deprivation during which he voided 1600 ml and lost 1.3 kg in weight. Plasma = 308 mOsm/kg; urine = 60 mOsm/kg.

(*d*) 50 ml of plasma from a male with pituitary diabetes insipidus (Schfdt) after 9 hr of complete fluid deprivation during which he voided 3800 ml and lost 3.9 kg. Plasma = 305 mOsm/kg; urine = 62 mOsm/kg).

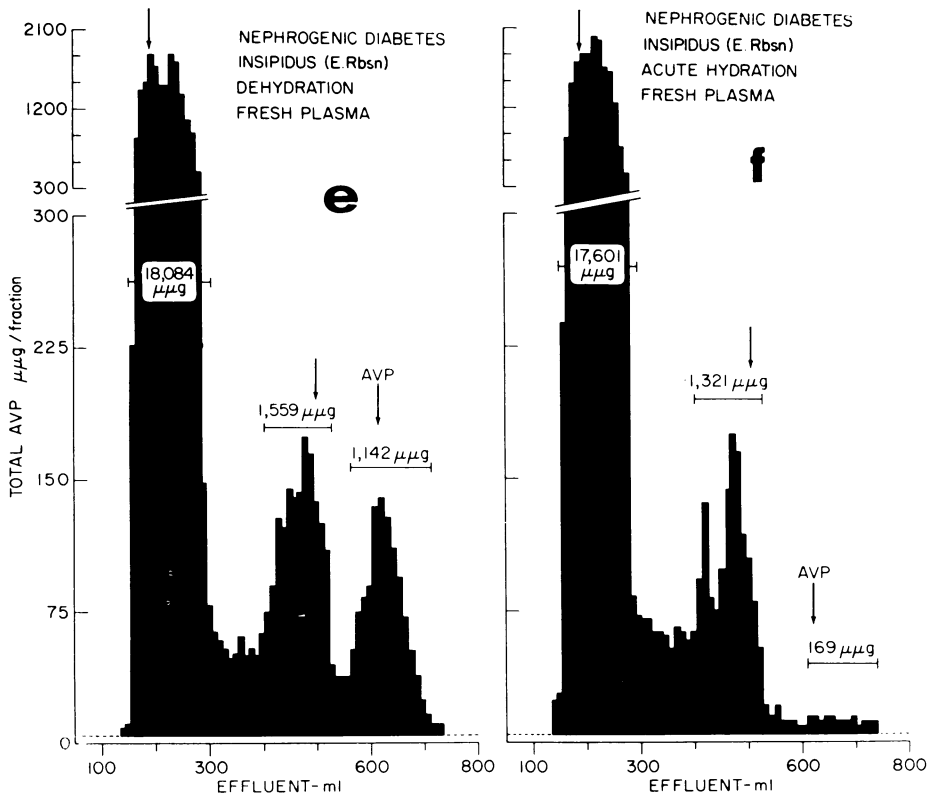


Fig. 1.—Vasopressin immunoreactivity in human plasma.

(*e*) 50 ml of plasma from a male with nephrogenic diabetes insipidus (E. Rbsn) after 10 hr of complete fluid deprivation during which he lost 5.2 kg. Plasma = 324 mOsm/kg; urine = 126 mOsm/kg.

(*f*) 50 ml of plasma from a male with nephrogenic diabetes insipidus (E. Rbsn) after he had ingested 8000 ml of water over a 90-min period. Plasma = 277 mOsm/kg; urine = 62 mOsm/kg.

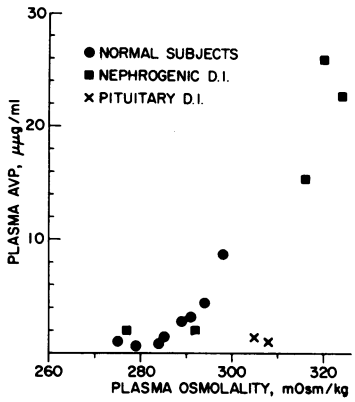


Fig. 2.—The relation of plasma osmolality to vasopressin concentration. Plasma vasopressin concentration, expressed as picograms of AVP immunoreactivity in peak III/ml plasma filtered, is plotted as a function of plasma osmolality for each of 15 plasmas from three normal adults (one male and two females), three patients with nephrogenic diabetes insipidus, and two patients with pituitary diabetes insipidus obtained after periods of controlled fluid intake. For plasmas with osmolalities in excess of 285 mOsm/kg, $r = 0.95$.

peak III immunoreactivity was very low, even when the plasma osmolality exceeded 300 mOsm/kg. The concentrations of immunoreactivity in peak I and peak II of plasma were unaffected by the state of hydration or by abnormalities in vasopressin function.

When pure AVP at physiologic concentrations was added to vasopressin-free plasma from a hydrated normal adult and filtered on Sephadex G-25, 75% of the AVP was recovered in a single peak identical with that of peak III (Fig. 3). Identical results were obtained when pure AVP was added to normal saline and filtered on the same column.

Plasma from a dehydrated normal adult was filtered on Sephadex G-25 and the fractions corresponding to peak III, free of salt, were pooled, lyophilized, reconstituted in a small volume, and compared with pure AVP. When filtered on Sephadex G-15 in 0.2 M acetic acid, peak III and pure AVP were indistinguishable (Fig. 4*a*). When injected into ethanol-treated rats,[6] peak III had 400 μU of antidiuretic activity per nanogram of immunoreactivity, which is about the same as that of pure AVP. However, when the peak III concentrate was immunoassayed over a 36-fold range of concentrations, its reactivity was similar to but not identical with that of the pure AVP (Fig. 5).

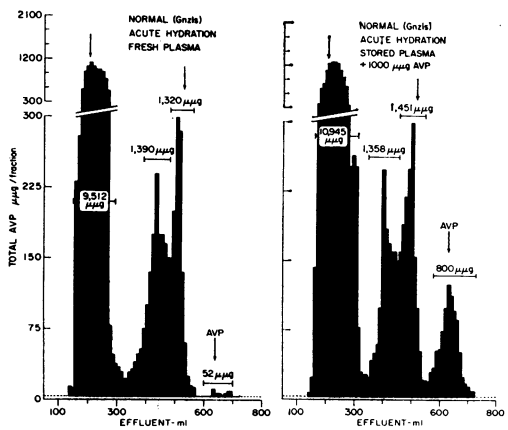


Fig. 3.—Recovery of vasopressin added to plasma. 450 ml of blood were drawn from a recumbent normal adult female following the administration of 1000 ml of water orally and 500 ml of normal saline intravenously over a 90-min period. 50-ml aliquots of the plasma were filtered on Sephadex G-25 (see *Methods*) before (*a*) and after (*b*) the addition of 1000 pg of pure AVP; 75% of the added hormone was recovered in the region of peak III with a peak coincident with that of endogenous peak III (see Fig. 1).

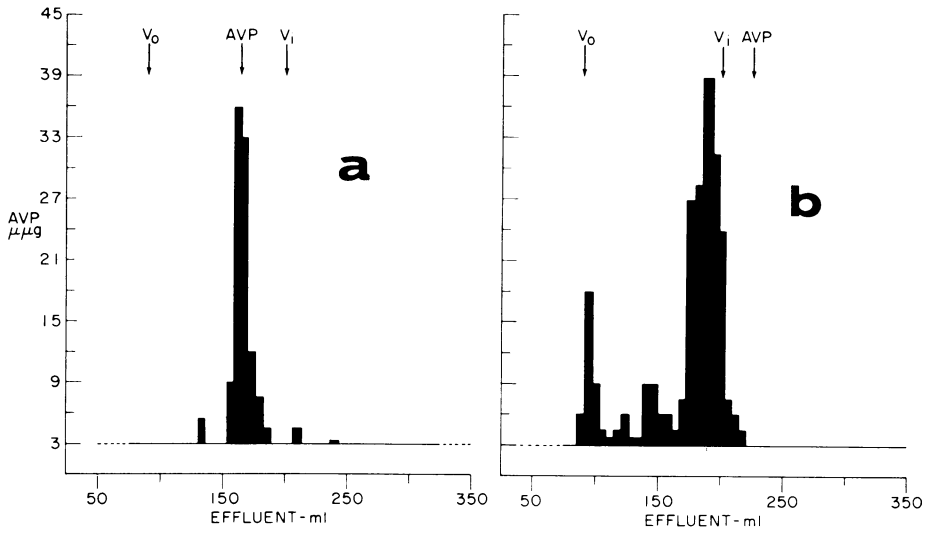


Fig. 4.—Repeat gel filtration of individual components of plasma immunoreactivity.

(*a*) 50 ml of plasma were filtered and assayed, and the fractions that corresponded to the third peak of immunoreactivity, free of plasma proteins and salt, were combined, lyophilized, and refiltered on a 2 × 94-cm column of Sephadex G-15 in 0.2 M acetic acid. The immunoreactivity was recovered (54% of the original) in a single discrete peak halfway between the internal and void volumes of the column (determined with serum albumin and sodium chloride). When pure AVP was filtered on the same column (also in absence of sodium chloride), it was recovered (60% of the original) at exactly the same position (*arrow*).

(*b*) Immunoreactive material of the second peak, including the plasma salt but not the protein, was prepared and refiltered as above. The immunoreactivity was recovered (46% of original) in four peaks, none of which corresponded to the elution position of pure AVP (*arrow*) when it was filtered on this column in the presence of comparable concentrations of sodium chloride.

A concentrate of peak II, which contained the plasma salt, reacted nonidentically with pure AVP (Fig. 5). When AVP^{125}-I was incubated with the peak II concentrate for 3 days at 4°C, only about 70% of the AVP^{125}-I was precipitated by the subsequent addition of an excess of rabbit anti-AVP; under identical conditions peak III concentrates produced no loss of immunoprecipitability of AVP-^{125}I. On filtration on Sephadex G-15 in 0.2 M acetic acid, peak II material yielded four peaks of immunoreactivity, none of which corresponded to the location of pure AVP when it was filtered in this system in the presence of a comparable concentration of sodium chloride (Fig. 4*b*).

A concentrate of peak I, which included the bulk of plasma proteins, reacted with anti-AVP in a fashion that was distinct from that of pure AVP or that of peak III (Fig. 5). When AVP-^{125}I was incubated with peak I and subsequently reacted with excess anti-AVP, only one third of the ^{125}I was in the precipitate. When filtered on Sephadex G-100 in 0.03 M ammonium acetate, the peak I concentrate yielded four peaks of immunoreactivity; the major peak corresponded to the void volume, two peaks appeared between plasma albumin and the salt, and one peak corresponded to the included volume. Peak I from plasma of a hydrated normal adult gave identical results.

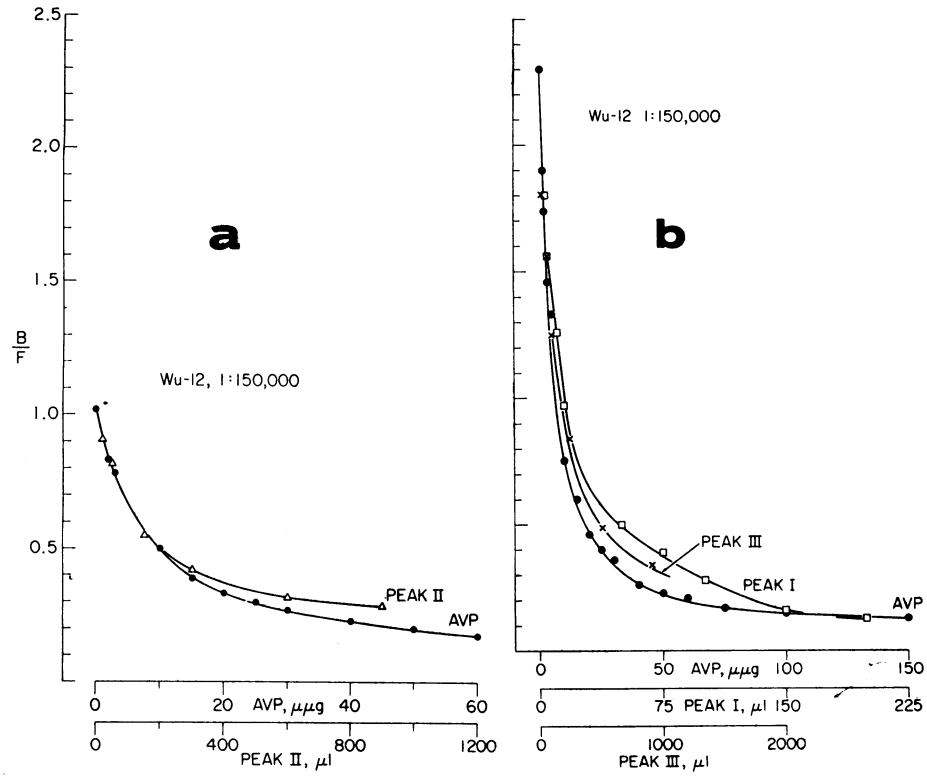


Fig. 5.—Immunological reactivity of plasma components. Plasma from a dehydrated adult was filtered on Sephadex G-25. Fractions corresponding to each peak of immunoreactivity were pooled, lyophilized, redissolved in diluent, and assayed against pure AVP. The bound/free of AVP-^{125}I is plotted against the concentration of pure AVP and against the volume of the plasma components. No correction was made for decreases in the immunoreactivity of the AVP-^{125}I after its exposure to peak I or peak II material (see *Results*).

Discussion. We have concluded that peak III of plasma immunoreactivity is endogenous circulating vasopressin. Peak III material was indistinguishable from pure AVP in two systems of gel filtration and, in limited studies, in its antidiuretic potency; its concentrations in plasma over a wide range agreed fully with established concepts of vasopressin physiology in normal and disordered states.

Physiologic amounts of AVP that were added to hormone-free plasma or to normal saline and carried through the entire procedure were reproducibly recovered in good yield. The effects of the ionic milieu on the adsorbtion of vasopressin to dextran gels will be reported in detail elsewhere[9] and are similar to effects on oxytocin and free tyrosine.[10,11]

The small difference in immunological reactivity between peak III and pure AVP is unexplained. Either substance could have been altered slightly during preparation or storage. Alternatively, the peak III concentrate may have been contaminated with small amounts of immunoreactive materials that are not vasopressin. Also, the persistence of minute amounts of immunoreactivity in

peak III of plasma from patients with pituitary diabetes insipidus or water-loaded normals is as yet unexplained. The difference in immunoreactivity between peak III and pure AVP appears only at high concentrations of hormone and therefore has little effect on the quantitation of circulating vasopressin.

The very close correlation between plasma osmolalities and AVP concentrations over a wide range of conditions attests to the precision of the methods as well as to the sensitivity of the osmoreceptors that control secretion of this hormone. It also suggests that other factors that influence vasopressin secretion were relatively unimportant in these studies. In particular, removal of up to 500 ml of blood from recumbent adults did not elevate the AVP concentration in the resultant plasma pool. The concentrations of plasma AVP, obtained by gel filtration and immunoassay, are in the same range reported by investigators who used other extraction procedures and biological assays.[12] Recently we have obtained reliable measurements of endogenous vasopressin with 5 ml of plasma; the effluent fractions that appeared after the peak of electroconductivity were pooled and lyophilized for assay in 1 ml.

In contrast to assays of insulin[13,14] and gastrin,[15] where additional immunoreactive components of plasma have been characterized, the source, nature, and significance of the vasopressin immunoreactivity in peaks I and II are unknown. In these and other studies we have been unable to detect vasopressin nonapeptide in these peaks, or to relate their concentration to vasopressin physiology. At least part, and possibly all, of the peak I and II immunoreactivities is due to damage to AVP-^{125}I, to plasma proteins that bind AVP-I, or to effects on antigen-antibody reactions rather than competition with AVP-^{125}I for binding to sites on AVP antibody. Gel filtration of canine plasma yielded peaks I and II at concentrations comparable to those found in plasma from humans.

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