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I. MEASUREMENT OF OXYTOCIN

Oxytocin: Clinical and Laboratory Studies, J.A. Amico and A.G. Robinson, editors

The radioimmunoassay of oxytocin: new developments

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Radioimmunoassay (RIA) of oxytocin provides a sensitive and specific method of measuring the small quantities of this hormone which are released in the circulation in response to physiological stimuli. However, the few research laboratories which measure oxytocin are not in agreement about the methodology of the assay or basal and stimulated levels in human plasma. Because the ability to measure oxytocin has been difficult, physiological roles for the hormone have been slow to unfold.

The methods of the RIA of oxytocin will be addressed in the following chapters. Certain steps of the RIA of oxytocin are considerations universal to all RIAs, viz. choice of the most appropriate reference standard, method of iodination, and most appropriate conditions to achieve equilibrium. Other adaptations in the RIA of oxytocin are made because oxytocin is a small peptide; for instance, the hormone must be coupled to a reagent to enhance antigenicity to produce antibodies, and is usually extracted from plasma to minimize background interference (Chard 1973; Kagan and Glick 1974). One problem unique to the measurement of oxytocin in the plasma of pregnant women is the potential destruction of the hormone in vitro by the cystine aminopeptidase (James et al. 1969; Vorheer and Kleeman 1980) which rises in late pregnancy. The recent findings from two laboratories, in Pittsburgh and Torrance, are presented in this chapter. The observations support the presence of a novel oxytocin-like peptide in human plasma, previously not described.

Prior to our ability to measure the neurohypophysial hormones, we isolated two specific human neurophysins and demonstrated independent release of the neurophysins in response to certain physiological and pharmacological stimuli (Robinson 1975). Nicotine-stimulated neurophysin (NSN, now termed vasopressin neurophysin), was found to be released at times of stimulated release of arginine vasopressin (AVP) (Husain et al. 1975). The other neurophysin, estrogen-stimulated neurophysin (ESN), was found to be elevated in response to administered estrogen (Robinson 1974), and elevated at mid-cycle during physiological increase in estrogen (Robinson et al. 1976).

The association of ESN with oxytocin was postulated because immunohistochemistry localized NSN in the cells of the hypothalamic nuclei which contain AVP, and ESN in the cells which contain oxytocin (Seif and Robinson 1978). In addition, it is now known that the precursor for oxytocin contains the sequence of both the hormone and the neurophysin as discussed in the chapter by Ivell and Richter in This Volume. When we developed a RIA for oxytocin, we found a parallel increase of oxytocin and ESN in human plasma in response to administered estrogen (Amico et al. 1981). The oxytocin response was described as specific because the oxytocin antiserum, Pitt Ab-1, detected appropriate changes in levels of oxytocin in individuals receiving an infusion of oxytocin and an increase in endogenous oxytocin in plasma in response to infant suckling during breast feeding (Amico et al. 1985).

A second antiserum to oxytocin, Pitt Ab-2, measured comparable increases in the levels of oxytocin in plasma during infusion studies and breast feeding. The Pitt Ab-2 RIA curve with biologic or synthetic standards of oxytocin was the same as that for Pitt Ab-1. However, the Pitt Ab-2 RIA measured no increase in the levels of oxytocin in plasma after administration of estrogen. Likewise, a third oxytocin antiserum used in the Torrance laboratory (Tor OT Ab) did not measure an increase in oxytocin in plasma in circumstances of estrogen hypersecretion (Leake et al. 1981). However, an arginine vasotocin (AVT) RIA system (Tor AVT Ab) used in the Torrance laboratory detected an increase in plasma AVT-like immunoreactivity after estrogen administration suggesting that an oxytocin/AVT-like immunoreactive material which rises in response to estrogen provocation was present in human plasma and was detected in certain RIAs for oxytocin.

Cross-reactivity studies of oxytocin antisera

The standard curve for oxytocin was prepared with USP-posterior pituitary reference standard (U.S. Pharmaceuticals, Rockville, MD) in potassium phosphate buffer (KPO_4), 0.01 M, pH 7.4, and 0.25% bovine serum albumin (BSA) and was identical at all points with synthetic standard (Syntocinon, Sandoz Pharmaceuticals). A dilution of antibody which bound 40% of iodinated oxytocin tracer was used and was 1:46,000 and 1:100,000 final dilutions for Pitt Ab-1 and Pitt Ab-2, respectively. The total volume of the assay mixture was 1 ml/tube. The AVT standard curve was prepared using synthetic AVT (206 vasopressor U/mg, Merck, Sharp and Dohme), and the Tor-AVT antiserum dilution was 1:70,000. Oxytocin was extracted from plasma with acetone and ether prior to RIA of plasma.

Standard curves were prepared in triplicate and the peptides listed in Table 1 were assessed for cross-reactivity with both Pittsburgh oxytocin antisera. Cross-reactivity of the following neurohypophysial peptides with

TABLE 1. *Cross-reactivity of Pitt Ab-1 and Pitt Ab-2*

CRP*	Quantity of CRP	Source of CRP	Pitt Ab-1 (%)	Pitt Ab-2 (%)
Oxytocin	0–100 μU per tube	Sandoz Pharmaceuticals	100	100
Vasotocin	0–10 ng per tube	ICN Pharmaceuticals	0.1	50
Arg** vasopressin	0–5000 μU per tube	Parke-Davis	0.2	0.2
Lys† vasopressin	0–5000 μU per tube	Parke-Davis	0.1	0.1
Tocinamide	0–56 pg per tube	Peninsula Laboratories	0.1	0.1
Pro-leu-gly (NH_2)	0–24 pg per tube	Peninsula Laboratories	0.1	0.1
Neurophysin	0–40 ng per tube	NIAMDD	0.1	0.1
Prolactin	0–40 ng per tube	NIAMDD	0.1	0.1
TRH	0–100 ng per tube	Abbott Laboratories	0.1	0.1
ACTH	0–10 ng per tube	Organon Laboratories	0.1	0.1
LHRH	0–100 ng per tube	Ayerst Laboratories	0.1	0.1

*CRP = cross-reacting peptide. **Arg = arginine. †Lys = lysine.

the Tor AVT Ab was also tested in the Torrance laboratory: oxytocin, AVP, mesotocin, valitocin, isotocin, glumitocin, aspargtocin, 8-glycine-oxytocin, pressinamide, tocinamide, and deamino-AVT. In all comparisons cross-reactivity was calculated as weight standard oxytocin or AVT/weight cross-reacting peptide or oxytocin or AVT at the 50% displacement point of the binding displacement curves.

No displacement of iodinated oxytocin was found with either Pitt Ab-1 or Pitt Ab-2 when assaying 100 μU AVP, 100 μU LVP, 2.0 ng DDAVP, 56 pg synthetic 'ring' or 'tail' of oxytocin, 40 ng of either human neurophysin, 40 ng prolactin, 100 ng TRH, 10 ng ACTH or 100 ng LHRH. A 50% cross-reactivity of AVT with Pitt Ab-2, but not with Pitt Ab-1, was found. The Tor AVT Ab had a 3% cross-reactivity with oxytocin.

Human studies

Oxytocin in plasma of breast-feeding women

The release of oxytocin in response to a known physiological stimulus was tested in 3 healthy women in established lactation. Blood was drawn prior to the start of infant suckling and every 3 min during 4 individual 21-min nursing periods in the 3 women. Both Pitt Ab-1 and Pitt Ab-2 detected a rise of oxytocin in response to infant suckling. The qualitative patterns of

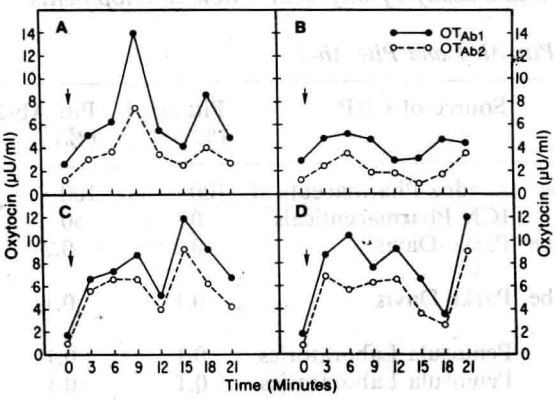

FIG. 1. *Oxytocin during breast feeding. Oxytocin was measured in plasma obtained from 3 women in established lactation prior to and every 3 minutes during 4 nursing periods (A through D), each of 21 minutes duration. During infant suckling, oxytocin was higher when measured with Pitt Ab-1 than with Pitt Ab-2. The arrows indicate the onset of infant suckling. (Reproduced from Amico et al. (1985), by courtesy of the Editors of the* Journal of Clinical Endocrinology and Metabolism.*)*

oxytocin were comparable with the 2 antisera, but oxytocin levels measured with Pitt Ab-1 were statistically greater ($p < 0.05$) at all times except basally (Fig. 1).

Basal levels of oxytocin in plasma of men and women

The mean $\pm$ SEM basal concentration of oxytocin in samples of plasma from normal men when measured with Pitt Ab-1 was 1.7 ± 0.09 μU/ml, a value significantly higher than the result using Pitt Ab-2, 0.8 ± 0.06 μU/ml, $p < 0.001$ (Table 2). In samples of plasma from normal women the mean $\pm$ SEM basal oxytocin measured with Pitt Ab-2 was 0.8 ± 0.09 μU/ml, significantly lower than the result using Pitt Ab-1 of 1.7 ± 0.07 μU/ml, $p < 0.001$ (Table 2).

Levels of oxytocin in plasma in response to administration of estrogen

Samples of plasma ($N = 80$) from women chronically using oral contraceptives had a mean Pitt Ab-2 oxytocin level of 0.8 ± 0.04 μU/ml, a value statistically similar to that in women not ingesting estrogen (Table 2). However, with Pitt Ab-1, the mean oxytocin level was 4.6 ± 0.5 μU/ml, a result significantly higher than that in women not ingesting estrogen ($p < 0.001$), and higher than the result measured with Pitt Ab-2 (Table 2).

Four women were given an oral contraceptive containing 100 μg mestranol

TABLE 2. *Concentrations of oxytocin in human plasma measured with 2 antisera to oxytocin*

	Pitt Ab-1 (μU/ml)	Pitt Ab-2 (μU/ml)
Women, basal	1.7±0.07	0.8±0.09
Men, basal	1.7±0.09	0.8±0.06
Women, chronic estrogen	4.6±0.5	0.8±0.04
Women, pre-acute estrogen	1.6±0.2	0.3±0.06
Women, post-acute estrogen	4.3±0.5	0.6±0.01
Women, pregnant	8.6±1.0	1.0±0.3
Men and women, chronic renal failure	12.9±1.5	2.5±0.1

daily for 3 days. The mean Pitt Ab-2 oxytocin prior to estrogen (0.3±0.06 μU/ml) was similar to that during administration of estrogen (0.6±0.01 μU/ml; Table 2). In contrast, the mean oxytocin Pitt Ab-1 value in the same women was significantly higher during estrogen than before estrogen treatment (4.3±0.5 *vs* 1.6±0.2 μU/ml, $p<0.001$; Table 2). The increase in oxytocin Pitt Ab-1 was not altered by pretreatment with oral medroxyprogesterone, 10 mg daily, for 5 days prior to administration of estrogen. The rise in oxytocin Pitt Ab-1 was also present in ovariectomized women who were given an oral dose of estrogen on the 5th to 7th days following hysterectomy and bilateral oophorectomy.

The response to larger doses of estrogen was tested. Three men given 25 mg diethylstilbestrol (DES) had a sustained rise of oxytocin with Ab-1, but not with Ab-2. Plasma from 3 additional men given DES was assayed for AVT and oxytocin and a significant increase in immunoreactive AVT, comparable to that of oxytocin with Ab-1 (Fig. 2), was found. No increase in Pitt Ab-2 was found.

Infusion studies

Both oxytocin antisera were tested for their ability to detect intravenously infused synthetic oxytocin (Pitocin, Parke-Davis). Four healthy, premenopausal women received oxytocin at an initial rate of 1 mU/min which was increased every 40 min by increments of 1 mU/min for 8 hours. The range was 1–12 mU/min. Blood was drawn before the start of the infusion and every 20 min during the infusion and oxytocin measured with Pitt Ab-1 and Pitt Ab-2. The relationship between the dose of oxytocin infused (mU/min) and the level of oxytocin in plasma (μU/ml) was linear when measured with both Pitt Ab-1 and Pitt Ab-2. A coefficient of correlation (R^2) was calculated by regression analysis using the concentration of oxytocin in plasma at 40 min of each dose of the infusion and was 0.75 for both antisera ($df=46$, $p<0.001$).

An infusion of synthetic oxytocin (Pitocin, Parke-Davis), 125 mU/min, was

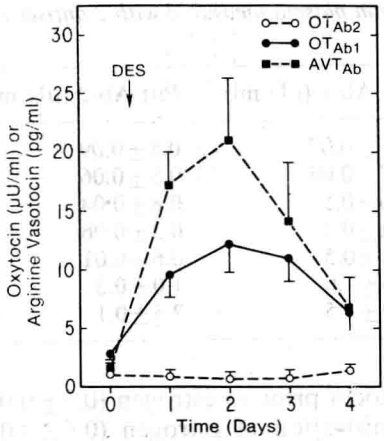

FIG. 2. *Acute administration of estrogen in men. Blood was obtained from 3 men prior to and daily for 4 days after a single oral dose of 25 mg diethylstilbestrol (DES). A significant increase in oxytocin (Pitt Ab-1) and AVT, but not oxytocin (Pitt Ab-2), was found. The increase was found by Day 1 and sustained for several days. A μU of oxytocin is equivalent to 2 pg oxytocin or AVT.*

administered for 60 min to 4 men on Day 1 and the infusion repeated in each man on the 3rd day of daily administration of one tablet of Ovulen. A placebo infusion of 0.9% saline was given for the 60 min prior to the oxytocin infusion. From a vein in the arm opposite to the site of infusion, blood was drawn every 15 min during both the saline and oxytocin infusions, and frequently after the oxytocin infusion was stopped. Oxytocin was measured using both Pitt Ab-1 and Pitt Ab-2.

Prior to infusion of oxytocin in 4 men, the mean oxytocin level with Pitt Ab-1 was significantly higher in the estrogen-treated than the non-estrogen-treated state (7.8 ± 0.1 μU/ml *vs* 2.9 ± 0.2 μU/ml, respectively, $p < 0.001$); but, oxytocin levels with Pitt Ab-2 were similar (0.80 ± 0.07 μU/ml *vs* 1.1 ± 0.14 μU/ml, pre- and post-estrogen, respectively). A plateau in plasma oxytocin was achieved by 30 min of the oxytocin infusion (as determined by both Pitt oxytocin assays) and the levels were not significantly different pre- and post-estrogen. The Pitt Ab-1 levels, but not Pitt Ab-2 values, were significantly higher ($p < 0.001$) after oxytocin infusion during the time of administered estrogen. The difference was not simply due to a higher basal Pitt Ab-1 value after administered estrogen because correcting each post-infusion Pitt Ab-1 value by subtraction of the mean basal oxytocin Pitt Ab-1 concentration from each post-infusion Pitt Ab-1 value resulted in significantly higher oxytocin levels with Pitt Ab-1 than Pitt Ab-2 ($p < 0.001$) at all times after estrogen. A longer half-life of oxytocin in plasma was measured with Pitt Ab-1 (26 min $\pm$ 5 SEM) when a subject was ingesting estrogen than during the basal

state (18 min ± 1.6, $p < 0.05$). The plasma half-life of oxytocin measured with Pitt Ab-2 was the same (15.3 min ± 1.1 SEM *vs* 15.6 min ± 0.4 SEM) before and during estrogen administration, respectively.

In vitro studies

In vitro effects of enzymes which degrade oxytocin and AVT

Fifty μU of oxytocin (USP Standard, U.S. Pharmaceuticals, Rockville, MD) were added to normal human plasma or KPO_4 buffer, pH 7.4. To these solutions were added: (a) synthetic ethinyl estradiol (Sigma Chemicals, St. Louis, MO) dissolved in ethyl alcohol and added at concentrations of 50 and 100 μg per ml of plasma; (b) thioglycolic acid (Sigma Chemicals, St. Louis, MO) dissolved in distilled H_2O to a concentration of 0.01 M and adjusted to pH 7.5 with NaOH; or (c) chymotrypsin (Sigma Chemicals, St. Louis, MO) dissolved in distilled water and added in a concentration of 125 μg per 100 μl of oxytocin-enriched buffer. Samples were incubated at 22°C, and at 5, 15, 30, and 60 min of incubation, aliquots were removed and proteins precipitated with 2 ml of acetone for assay with Pitt Ab-1, Pitt Ab-2 and Tor AVT Ab. Incubations with chymotrypsin were also conducted for 1, 4, and 20 hours after mixing. In control studies, normal human plasma or buffer enriched with 50 μU of synthetic peptide per ml of plasma or buffer were similarly treated with an equal volume of either normal human plasma or buffer, or the diluent for the specific test substance. In another set of experiments, pregnancy plasma from a woman beyond the 36th week of pregnancy was mixed 1:2 (vol:vol) with 50 μU oxytocin per ml of KPO_4 buffer, pH 7.4, and incubated at room temperature for 0, 5, 15, 30 and 60 min.

Pooled plasma from normal women who were taking chronic estrogen in the form of oral contraceptives was enriched with 50 μU oxytocin per ml plasma and incubated with an equal volume of either additional pooled plasma from normal subjects or plasma from a woman in the late stages of the 3rd trimester of pregnancy. Unenriched pooled plasma from the women ingesting estrogen was also mixed with an equal volume of plasma from the same pregnant woman. Plasma was incubated at room temperature for 0, 5, 15, 30, and 60 min. At the end of each incubation period, aliquots of the test mixture were removed and extracted with acetone for assay with Pitt Ab-1 and Pitt Ab-2. The oxytocin or AVT level was expressed at each time as the percent of the total oxytocin or AVT present at the start of each incubation (time zero).

The recovery of synthetic oxytocin added to normal human plasma was unaffected by addition of ethinyl estradiol. Recovery of immunoreactive oxytocin added to normal human plasma or buffer in the presence of pregnancy plasma, thioglycolic acid, or chymotrypsin decreased with time of

incubation. The recovery of oxytocin with Pitt Ab-1 was not greater than with Pitt Ab-2. No loss of immunoreactive oxytocin was found in control experiments. No measurable AVT was found during incubation of synthetic oxytocin with chymotrypsin or thioglycolic acid. A progressive decrease in the recovery of oxytocin added to pooled normal plasma or plasma from an individual given estrogen occurred with increasing time of incubation with pregnancy plasma. The immunoreactive oxytocin Pitt Ab-1 in the plasma of an individual given estrogen was not changed after incubation with pregnancy plasma.

Preparation of plasma for high pressure liquid chromatography

Fifteen to 20 ml of plasma was aliquoted to 1.0 ml fractions and each ml extracted with acetone and ether, dried, and the residue reconstituted in 100 µl KPO_4 buffer, pH 7.4, and pooled to a volume of 1500 µl. The fraction was applied to an octadecasilyl silica (SEP-PAK C_{18}) reversed-phase cartridge (Waters Associates, Milford, MA) pre-washed with 5 ml of isopropanol and 10 ml deionized H_2O. The column was washed 3 times with 10 ml of 0.01 M distilled trifluoroacetic acid (TFA) (Fisher Scientific, Pittsburgh, PA), the sample eluted with 3 ml each of 25%, 50% and 75% acetonitrile CH_3CN (Waters Associates) in 0.01 M TFA, dried, reconstituted in 0.01 M TFA, and an aliquot applied to a C_{18} µ Bondapak Column (Waters Associates) for reverse phase high pressure liquid chromatography (HPLC). Preparation of plasma in this manner results in a greater than 200-fold decrease in protein with an efficiency of recovery of the peptide of interest of 75–80%.

HPLC of plasma

The HPLC solvent system was (pump A) 0.01 M TFA, and (pump B) 0.01 M TFA mixed 1:1 by volume with CH_3CN (Waters Associates, Milford, MA). Pump B was run over a linear gradient of 20–50% per 15 min at 2 ml/min at 22°C. One-minute fractions were collected for 1 min, air-dried, and reconstituted in KPO_4 and aliquots assayed for oxytocin, using both antisera, AVT, AVP and both human neurophysins.

Peak immunoreactivity of synthetic oxytocin, AVP, and AVT are shown in the top graph of Figure 3. Concentrated plasma from an individual during an infusion of synthetic oxytocin had peak immunoreactivity for oxytocin in the fraction co-eluting with the peak immunoreactivity for synthetic oxytocin (Fig. 3) and was detected by both antisera to oxytocin but not by the AVT antiserum. Plasma from an individual during administration of estrogen contained a material detected as immunoreactive oxytocin with Ab-1 and as immunoreactive AVT (Fig. 3). The material did not co-elute with synthetic AVT, oxytocin or AVP. Because of the close elution of the material to synthetic oxytocin, further studies were done.

Concentrated plasma from this individual was applied to the HPLC

column either alone or as a mixture with synthetic oxytocin and samples were collected at 20-sec intervals between 13 and 20 min of the HPLC program. Two peaks of immunoreactivity in the plasma from the individual administered estrogen were detected by Ab-1 and by the antibody to AVT, but not by

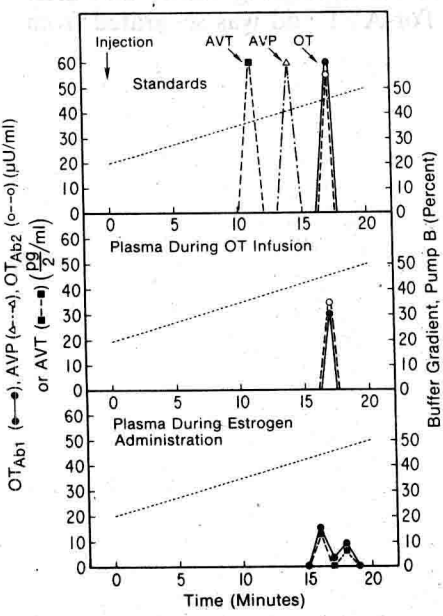

FIG. 3. *HPLC separation. HPLC was performed as described in the text. Aliquots were assayed using Pitt Ab-1, Pitt Ab-2, Tor-AVT and Pitt-AVP (100 μl each). Synthetic oxytocin, AVT, and AVP each at a concentration of 50 μU per 25-μl samples size were applied to the column and 1-min eluates were collected for 20 min and prepared for RIA. Synthetic AVT, AVP and oxytocin were detected by RIA in eluates collected at 11, 14 and 17 min of the program, respectively (top panel). Recognition of synthetic oxytocin was qualitatively and quantitatively similar for both antisera to oxytocin. Fifteen ml of plasma from an individual during administration of 25 mg DES and from an individual during infusion of synthetic oxytocin were applied to the HPLC column. The concentrated plasma from an individual during an infusion of synthetic oxytocin (middle panel) demonstrated peak oxytocin immunoreactivity in the fraction co-eluting with synthetic oxytocin. The immunoreactive oxytocin was detected by Pitt Ab-1 (●–●) and Pitt Ab-2 (○--○), but not by Tor-AVT. Concentrated plasma from an individual during administration of estrogen (lower panel) contained a material detected as oxytocin with Pitt Ab-1 (●–●) and as AVT with Tor-AVT (■ -- ■), but not as oxytocin with Pitt Ab-2 (○--○). The material did not co-elute with standard preparations of AVT, oxytocin or AVP. The legend on the right of the graph represents the buffer gradient expressed as a percent of pump B. (Reproduced from Amico et al. (1985), by courtesy of the Editors of the* Journal of Clinical Endocrinology and Metabolism.*)*

Ab-2 (Fig. 4). The peaks eluted before and after the peak of synthetic oxytocin. Synthetic oxytocin added alone to the column eluted as a single peak measured by Ab-1 and Ab-2, but not by the antibody to AVT. A mixture of plasma with synthetic oxytocin is shown in Figure 4. The peak recognized by Pitt Ab-1 and Pitt Ab-2 elutes in a position corresponding to synthetic oxytocin (Fig. 4). The immunoreactivity eluting before and after this peak was detected by Pitt Ab-1 and Tor-AVT and was separated from synthetic oxytocin (Fig. 4).

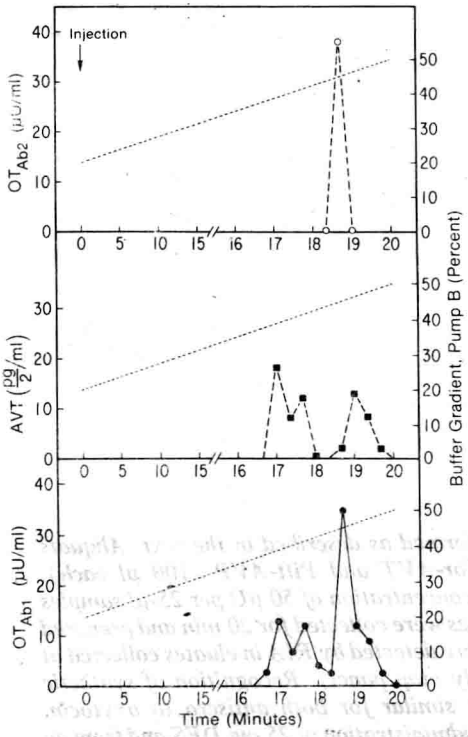

FIG. 4. *HPLC of a mixture of synthetic oxytocin and plasma from an individual during administration of estrogen. Seventy-five μl of the concentrated material from an individual during administration of estrogen was enriched with 10 μl of synthetic oxytocin (50 μU/25 μl) and the mixture was applied to the HPLC column. Synthetic oxytocin was detected by Pitt Ab-1 (lower graph) and Pitt Ab-2 (upper graph) but not by Tor-AVT. Synthetic oxytocin was detected as a single peak of immunoreactivity. The immunoreactivity detected by Pitt Ab-1 (lower graph) and by Tor-AVT (middle graph) was separated from synthetic oxytocin and is shown as 2 peaks of immunoreactivity eluting before and after synthetic oxytocin. (Reproduced from Amico et al. (1985), by courtesy of the Editors of the* Journal of Clinical Endocrinology and Metabolism.*)*

Plasma was obtained from 2 other individuals given estrogen and reverse phase HPLC separation of the plasma performed. One of the individuals had 2 peaks of immunoreactivity — one eluting before and one after synthetic oxytocin. In the other individual only one peak of immunoreactivity was found and eluted after the synthetic oxytocin. Thus, in all individuals an additional peak(s) of immunoreactivity is present and the peak(s) elute separately from synthetic oxytocin. In some individuals 2 peaks are present whereas in others only one peak is present.

Chronic renal failure

Because levels of the estrogen-stimulated neurophysin (ESN) rise in parallel with the oxytocin-AVT-like peptide, other conditions in which ESN was found to be increased were studied. We previously reported elevated levels of ESN in the plasma of individuals with end-stage renal failure (Amico et al. 1979). Levels of estrogen were not elevated and no correlation was found between ESN and estrogen in men or women.

Nine patients undergoing hemodialysis and 8 patients undergoing peritoneal dialysis had measurement of oxytocin (using Pitt Ab-1 and Pitt Ab-2), ESN, and AVT in plasma obtained prior to dialysis. Oxytocin Pitt Ab-1 immunoreactivity, 12.9 ± 1.5 μU/ml was significantly higher, $p < 0.001$, than oxytocin Pitt Ab-2 immunoreactivity, 2.5 ± 0.1 μU/ml (Table 2). AVT was accordingly increased, 32.1 ± 6.75 pg/ml. Immunoreactive levels of oxytocin measured with Pitt Ab-1 and ESN 13.5 ± 4.0 ng/ml were significantly higher, $p < 0.001$, in plasma of dialysis patients than in plasma of individuals with normal renal function. ESN was correlated with AVT ($R^2 = 0.81$, $p < 0.01$) and with oxytocin Pitt Ab-1 ($R^2 = 0.80$, $p < 0.001$), but not oxytocin Pitt Ab-2.

Plasma pooled from 6 of the patients with renal failure was prepared (as described above) and applied to the HPLC reverse phase column. One-minute fractions were collected between 1 and 12 min and every 20 sec between 13 and 20 min. Pooled plasma from individuals with renal failure had a peak of immunoreactivity detected by Pitt Ab-1 and Tor-AVT but not by Pitt Ab-2. The peak did not co-elute with synthetic oxytocin, AVT, or AVP. A mixture of renal failure plasma with synthetic oxytocin confirmed that the material was not oxytocin. Synthetic oxytocin was detected by Pitt Ab-1 and Pitt Ab-2, and the second peak of immunoreactivity, eluting after the oxytocin standard, was detected only by Pitt Ab-1 and Tor AVT Ab.

Pregnancy

Pregnancy is a physiological state of estrogen dominance and levels of ESN are high. Oxytocin in plasma from pregnant women was 8.6 ± 1.0 μU/ml