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Immunocytochemical Localization of Oxytocin and Neurophysin in Human Corpora Lutea

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ABSTRACT **Corpora lutea, corpora albicantia, and ovarian stroma from normal human premenopausal ovaries were examined for the presence of oxytocin and neurophysin by using highly specific antisera and peroxidase-antiperoxidase light-microscopic immunohistochemistry. Oxytocin and neurophysin immunoreactivity was found in some but not all cells of the corpora lutea obtained on days 19 to 24 of the menstrual cycle. Stromal tissue and corpora albicantia did not give a positive reaction for either of these peptides, and negative results were also obtained with corpora lutea of mid- and term-pregnancy and preovulatory follicles. Specificity of the immunohistochemical reaction was confirmed by immunoabsorption tests. The specific localization of immunoreactive oxytocin and neurophysin in corpora lutea of the human menstrual cycle directly demonstrates the presence of oxytocin- and neurophysin-positive cells within the human corpus luteum.**

INTRODUCTION

Recently we have demonstrated (Khan-Dawood and Dawood, 1983) that the neuropeptide oxytocin is present in higher quantities in human corpus luteum tissue than in the peripheral circulation, as determined by a sensitive and specific radioimmunoassay. To evaluate whether all cells of the human corpus luteum demonstrate the presence of neurohypophysial peptides, we performed studies to localize oxytocin and neurophysin within the luteal cells by using immunohistochemistry which employs the peroxidase-antiperoxidase (PAP) staining technique and light microscopy.

MATERIALS AND METHODS

Ovarian tissues were obtained from women undergoing gynecological surgery or repeat caesarean sections and tubal ligations. Each patient gave informed consent prior to surgery, and the decision to remove the entire ovary or to perform lutectomy was made by the operating surgeon based on clinical considerations. The different tissue types—stroma, corpora lutea, corpora albicantia, and follicles—were dissected within 10 min of removal of the ovary from the patient. Each ovarian sample was evaluated by the surgical pathologist for histological identification and was dated by endometrial histologic dating by the criteria of Noyes et al. (1950) and corpus luteum dating by the criteria of Corner (1956). All tissues obtained were histologically normal. Each tissue was immediately rinsed in normal saline at 4°C and fixed in Bouin's solution for 48 hr at 4°C. The tissues were then dehydrated and embedded in Surgipath paraffin (Surgipath Medical Industries Inc.). Tissue sections of 4 μm were cut on a Spencer "820" microtome and mounted on chrome alum gelatinized slides. The tissue was deparaffinized by placing in a 45°C oven for 30 min, followed by toluene, ethanol, and finally distilled water washing. The tissue was then washed with 0.05 M Tris-HCl buffer, pH 7.6, supplemented with 0.1% Triton X-100 (Sigma). The sections were incubated for 30 min at room temperature with 20% normal goat serum diluted in Tris-HCl buffer. The next incubation was with 1) the primary oxytocin antiserum (specific antiserum raised in rabbits immunized against oxytocin conjugated to BSA) (Dawood et al., 1978), or 2) nonimmunized normal rabbit serum, or 3) antisera preabsorbed with excess synthetic oxytocin (500 μg/ml oxytocin, Sandoz). The specificity of staining was also evaluated for both oxytocin and neurophysin antisera by the addition of 2.0 mg/ml of bovine serum albumin 48 hr before use. The primary antiserum and normal rabbit serum were used at a dilution of 1/100 in Tris-HCl buffer with 3% normal goat serum added. The incubation time was 1 hr at room temperature followed by 16 hr at 4°C. All incubations were carried out in a moist chamber. The indirect method of Sternberger (1979) employing peroxidase-antiperoxidase (PAP) with 3.3′ diaminobenzidine (Sigma) as a chromagen was employed to localize the neuropeptide. Incubations with the second antibody, goat antirabbit IgG (Pel-Freeze) in a dilution of 1/50 and substrate, rabbit PAP (Pel-Freeze) diluted 20 μg/ml, were for 1 hr each at room temperature. The 3.3′ diaminobenzidene tetrahydrochloride (Sigma) was dissolved at 0.5 mg/ml in 0.05 M Tris-HCl, pH 7.6, containing 3.3 μl of 3.0% hydrogen peroxide per ml. Alternate sections were counterstained with Mayer's hematoxylin (Sigma) or Mayer's hematoxylin and eosin (Orange G-6, Lerner Laboratories). The same procedure was employed for immunocytochemical staining for neurophysin I with a specific estrogen-stimulated neurophysin antibody raised in rabbits (Zimmerman et al., 1975). The neurophysin antiserum was used at a dilution of 1/2,500 in Tris-HCl buffer with 3% normal goat serum. For preabsorption of neurophysin antisera, 200 μg/ml of antigen was used (Sigma). A rat hypothalamus was used

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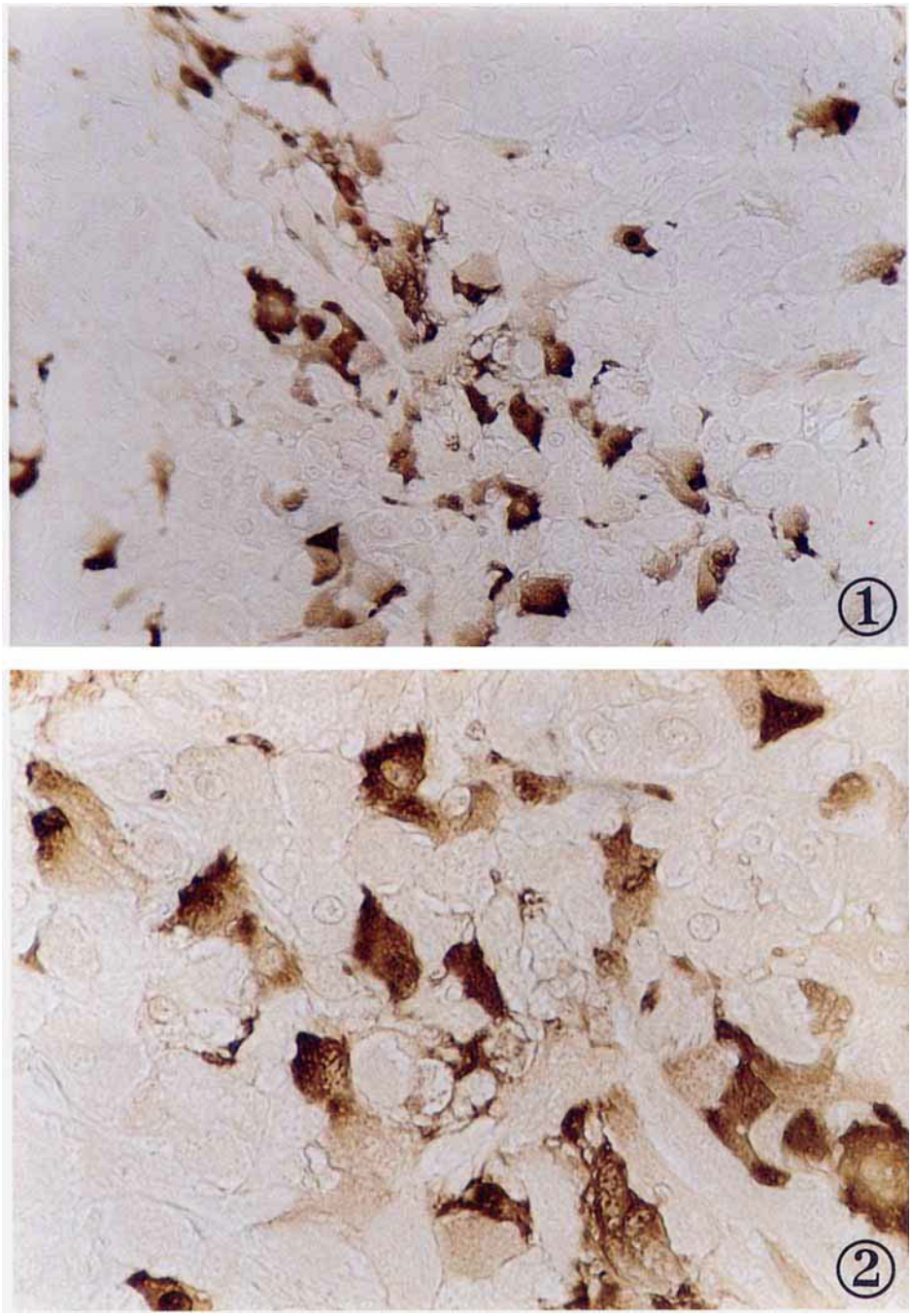
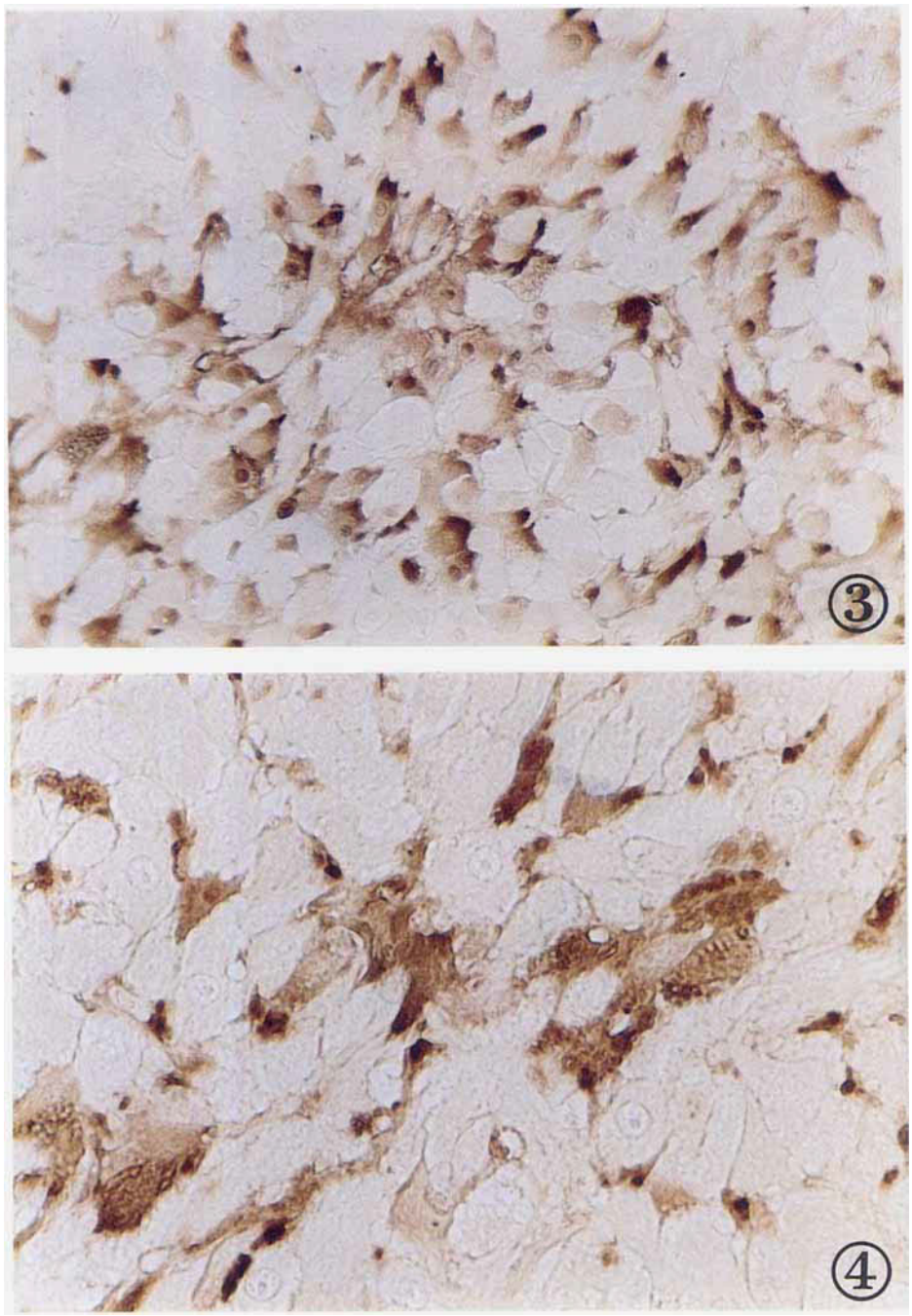


Fig. 1, 2. Photomicrograph of section of human corpus luteum from the midluteal phase (day 22 of the menstrual cycle) showing the presence of immunoreactive oxytocin by immunocytochemistry employing the peroxidase-antiperoxidase (PAP) technique. The antiserum for oxytocin was raised in rabbits immunized with oxytocin conjugated to bovine serum albumin and showed less than 0.02% cross-reaction with arginine-vasopressin, lysine-vasopressin, arginine vasotocin, angiotensin, and gonadotropin-releasing hormone. Note that oxytocin was localized predominantly in the cytoplasm of luteal cells which appear large. The nuclei of the cells show little staining for oxytocin and were positive in fewer cells. 1, × 200; 2, × 400.

as a serum control for both antisera and was treated identically to the other tissues.

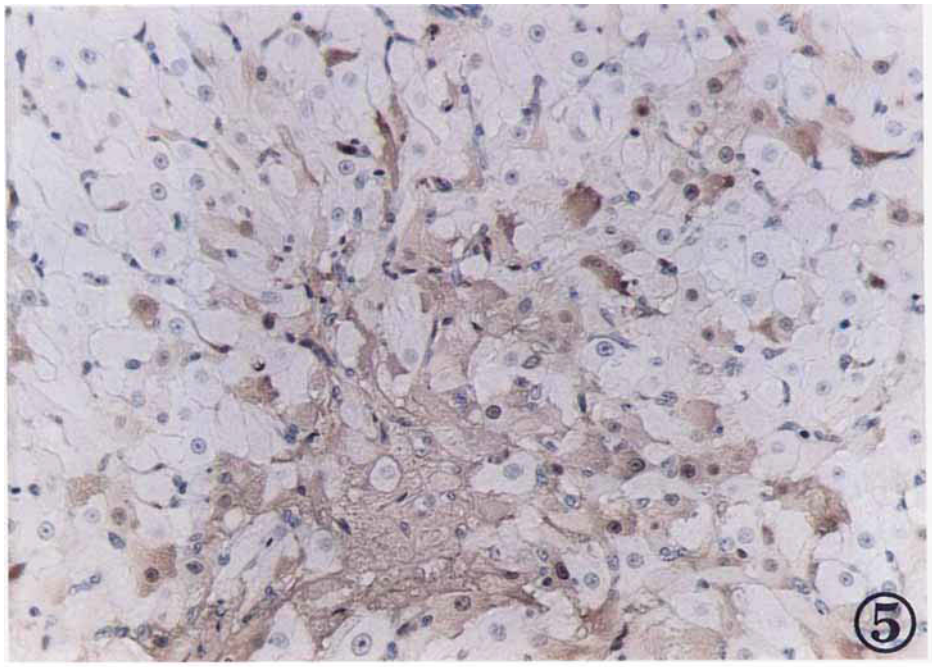
RESULTS

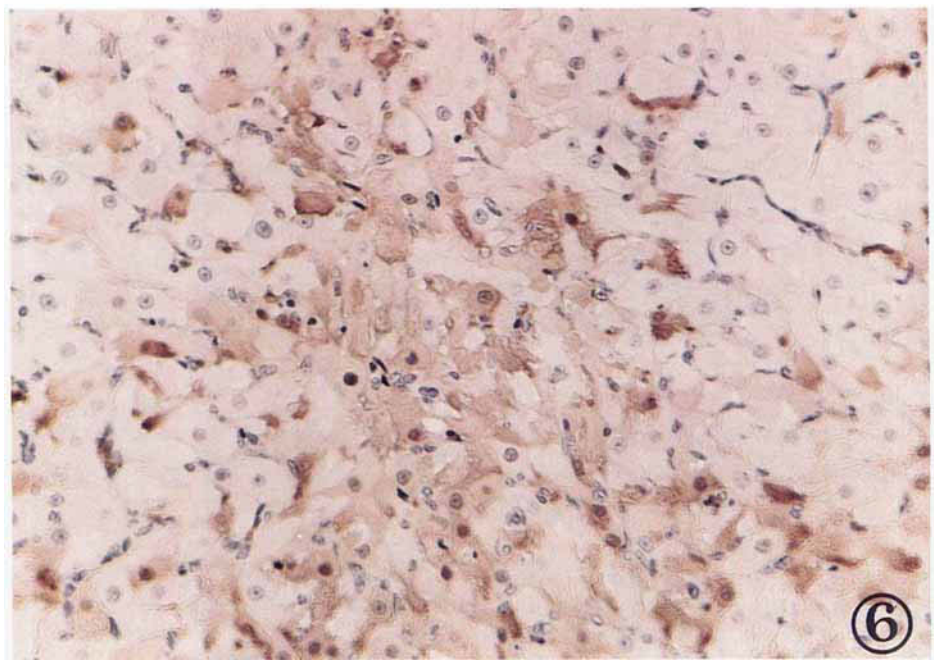
With both the oxytocin and neurophysin antisera (Figs. 1–4), specific staining was seen in all six of the corpora lutea obtained on days 19 to 24 of the menstrual cycle. The cells that stained for the neurohypophysial peptides are dispersed throughout the tissue (Figs. 1, 3) and cannot be differentiated from the surrounding cells that did not show a positive reaction under these conditions. As seen in Figures 5 and 6, however, counterstaining with hematoxylin indicates that the immunoreactivity is localized mainly in the cytoplasm of the cells, and the



Figs. 3, 4. A section of human corpus luteum showing positive staining for neurophysin with peroxidase-antiperoxidase technique. 3, × 200; 4, × 400.

stained areas have a granular appearance (Figs. 2, 4). The immunospecific staining was absent when the antisera were preabsorbed with excess antigen (Fig. 7) or the primary antisera was replaced with normal rabbit serum (Fig. 8). A corpus luteum obtained on day 26 of the menstrual cycle and six corpora albicantia were negative for both peptides. Ovarian stroma (Fig. 9) from the ovaries, both of cycling women and of a pregnant woman, and preovulatory follicles and corpora lutea obtained from mid- and term-pregnancy (Fig. 10), did not stain for either oxytocin or neurophysin (Table 1). Rat hypothalamic tissue which was used as a serum control shows the presence of oxytocin (Figs. 11, 12) and neurophysin in the perikarya and axonal processes.

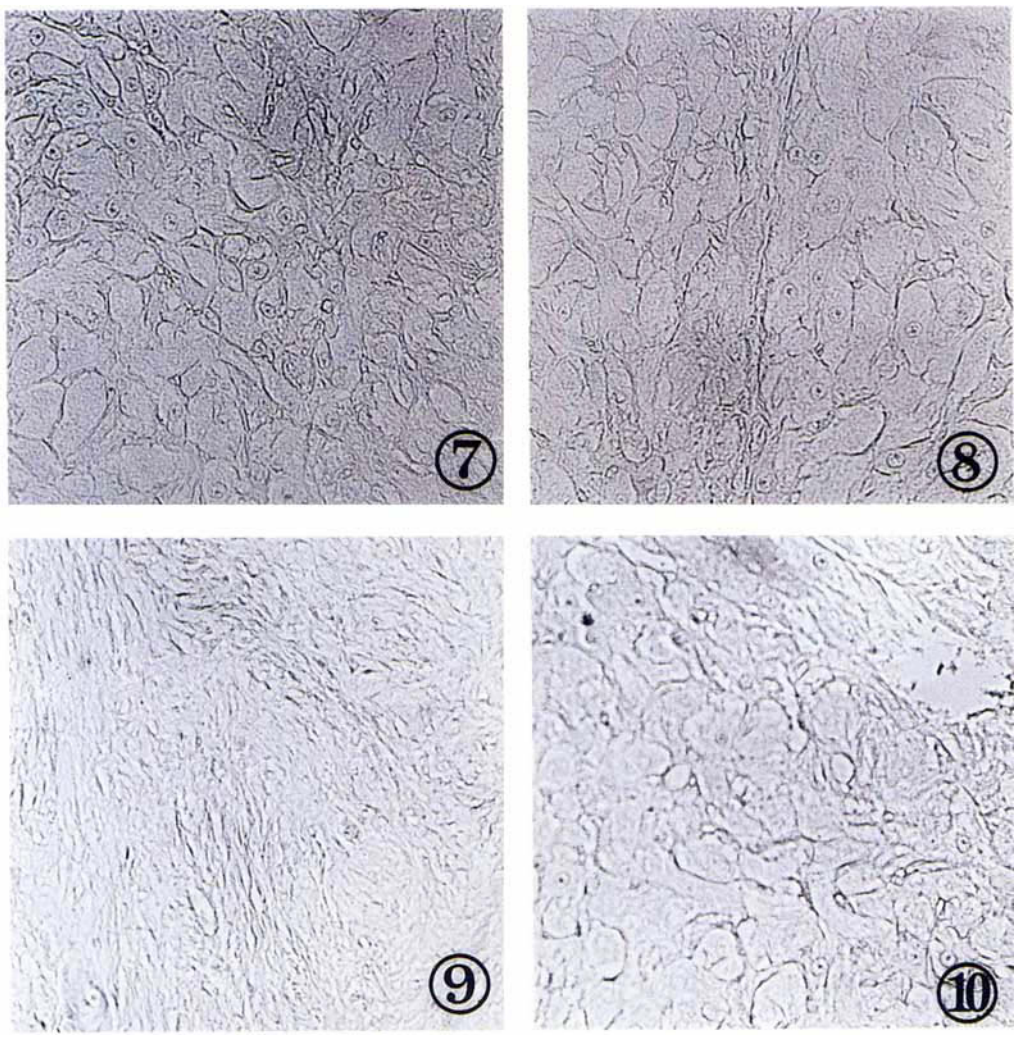




Figs. 5, 6. Photomicrographs of sections of human corpus luteum from the mid luteal phase (day 22 of the menstrual cycle) stained by the peroxidase-antiperoxidase technique for oxytocin (Fig. 5) and neurophysin (Fig. 6) and counterstained with hematoxylin. The immunoreactivity is localized as granular material predominantly in the cytoplasm of the luteal cells which are stellate in appearance. × 200.

DISCUSSION

The present study demonstrates the presence of immunoreactive neurophysin in human corpora lutea and the localization of both oxytocin and neurophysin within the luteal cells of the human corpus luteum of the menstrual cycle. The immunocytochemical localization of oxytocin in the human corpus luteum confirms previous data obtained by radioimmunoassay and high-pressure liquid chromatography (Khan-Dawood and Dawood, 1983; Wathes et al., 1982; Dawood and Khan-Dawood, 1986). As previously shown by radioimmunoassay, immunoreactive oxytocin cannot be localized in the stroma, corpora albicantia, and corpora lutea of pregnancy. Preovulatory follicles also do not stain positive for either oxytocin or neurophysin. Only in two nonprimate species have these peptides been shown to be present immunocytochemically. In the sheep corpus luteum



Figs. 7–10. Figure 7. Photomicrograph of section of human corpus luteum from the midluteal phase (day 22 of the menstrual cycle) showing absense of immunoreactivity when the oxytocin antiserum was preabsorbed with synthetic oxytocin in the PAP technique. × 200.

Figure 8. A similar section shows no immunoreactivity within luteal cells when normal rabbit serum was substituted for the oxytocin antiserum in the PAP technique.

Figure 9. Human ovarian stromal tissue shows no oxytocin immunoreactivity. Figure 10. Similarly, a section of corpus luteum from a normal term pregnancy also shows no reactivity when incubated with oxytocin antiserum employing the PAP techniques.

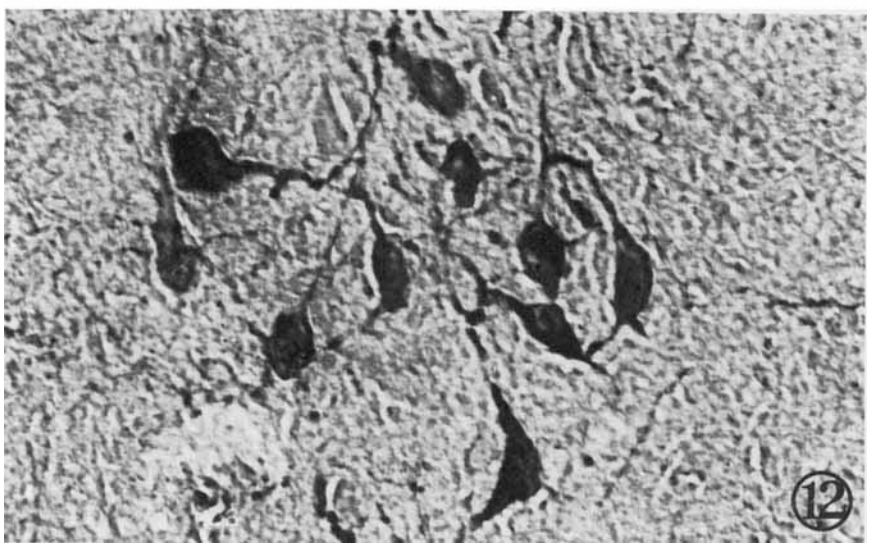
(Watkins, 1983; Theodosis et al., 1986; Sawyer et al., 1986) and the bovine corpus luteum (Guldenaar et al., 1984; Kruip et al., 1985), both oxytocin and neurophysin are present in the large cells. Both human (Khan-Dawood and Dawood, 1983) and bovine corpora lutea (Guldenaar et al., 1984) obtained during pregnancy had *immunoreactive* oxytocin concentrations which were considerably lower than those in the corpora lutea of the menstrual cycle as determined by radioimmunoassay. Neither oxytocin nor neurophysin could be detected in the corpus luteum of pregnancy by the specific immunohistochemical technique employed in this study because of the very low concentrations present. Similarly, we are unable to demonstrate a positive reaction for oxytocin in corpora albicantia and ovarian stroma by immunohistochemistry. This is consistent with our expanded radioimmunoassay data, in which the mean oxytocin concentration in corpora albicantia was 1.7 ± 0.2 ng/gm wet weight (± standard error of the mean) and was undetectable in the stroma, but was 30.8 ± 0.9 ng/gm wet weight in the corpora lutea of the midluteal phase (Dawood and Khan-Dawood, 1986). The tissues that are negative for oxytocin do not show the presence of neurophysin either. When alternate serial sections were stained for both peptides, however, oxytocin and neurophysin were localized in the same cells.

That the positive staining is immunospecific for both peptides is strongly supported by the absence of immunocytochemical staining when the antisera were preabsorbed with excess standard oxytocin or neurophysin or when normal rabbit serum was used as the primary antiserum. Furthermore, the similar positive reactions obtained in rat hypothalamic tissue serve as a method as well as tissue control. In the human corpus luteum, the immunoreactive oxytocin and neurophysin are localized predominantly in the cytoplasm of only some luteal

TABLE 1. Immunoperoxidase staining of oxytocin (OT) and neurophysin (NP) in the ovarian tissue of nonpregnant and pregnant women

Physiological state	Type of tissue	Result	
		OT	NP
Nonpregnant (day of cycle)			
1–12	*Corpora albicantia*	– (6)[1]	– (6)
19–24	Corpora lutea	+ (6)	+ (6)
26–30	Corpora lutea	– (1)	– (1)
1–30	Stroma	– (13)	– (13)
4–12	Preovulatory follicle	– (3)	– (3)
Pregnant (gestation age) in weeks			
16	Corpora lutea	– (1)	– (1)
38–39	Corpora lutea	– (2)	– (2)
16	Stroma	– (1)	– (1)

[1]Numbers in parenthesis indicate number of specimens.



Figs. 11, 12. Photomicrograph of section of rat hypothalamic tissue showing the presence of immunoreactive oxytocin in the perikarya and axonal processes by the PAP immunocytochemical technique, employing a specific antiserum for oxytocin. This section was used as a method and tissue control for the presence of immunoreactive oxytocin. 11, × 200; 12, × 400. All × 200.

cells which appear large, and the immunocytochemically stained areas appear granular. While the bovine (Parry et al., 1980) and ovine (Fitz et al., 1982) corpora lutea have large and small luteal cells and both oxytocin and neurophysin have been localized only in the large luteal cells which are steroid-producing, the presence of these cell types in human corpus luteum is yet to be established. Based on tissue sections alone, it is not possible to conclude that these peptides are localized in large luteal cells. Nonetheless the positive immunoreactivities for oxytocin and neurophysin are within the luteal cells of the human corpus luteus which also produce steroid hormones such as estrogen and progesterone.

The demonstration of both oxytocin and neurophysin within the human corpus luteum suggests that the tissue may have the capacity to synthesize these peptides. In the bovine corpus luteum (Swann et al., 1984; Ivell and Richter, 1984) as in the hypothalamus (Gainer, 1983), oxytocin and neurophysin I are produced from the same messenger RNA molecule, and oxytocin is cleaved from the carrier protein after translation.

The localization of oxytocin and its associated protein, neurophysin, within the luteal cells raises the question as to its role in the corpus luteum. Based on *in vitro* studies with human corpora lutea (Tan and Tweedale, 1982), luteal tissue oxytocin is most likely subserving a paracrine role in regulating the life span of the corpus luteum through luteal tissue steroidogenesis and/or prostaglandin production or release (Dawood and Khan-Dawood, 1986). Further studies are needed to elucidate the role and mechanism of action of oxytocin in regulating the *human* corpus luteum, since the mechanisms involved in small animals are not always operative in the human female.

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