Detection of Prolactin Messenger RNA in Rat Anterior Pituitary by In Situ Hybridization

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The effects of chronic diethylstilbestrol treatment on rat prolactin mRNA was analyzed by in situ hybridization histochemistry. Forty-day-old female rats were treated with 10 mg diethylstilbestrol in Silastic tubes for 3, 6, and 9 weeks. Estrogen treatment for 9 weeks increased pituitary wet weight (51.6 ± 2.4 versus 7.9 ± 0.31 mg for controls), serum prolactin (4155 ± 571 versus 47.1 ± 8.9 ng/ml for controls), and the percentage of immunoreactive prolactin cells (69% ± 3% versus 34% ± 2% for controls). In situ hybridization studies showed an increase in rat prolactin mRNA with increasing duration of estrogen treatment. After 9 weeks of estrogen treatment, there was a 2.3-fold increase in rat prolactin mRNA. ^{3}H-cDNA was distributed diffusely throughout the anterior pituitary in both normal and hyperplastic pituitaries.

There were no separate foci of adenomatous pituitary with increased labeling or with increased immunoreactive PRL cells. Although transplantable pituitary MtT/W15 tumors secreted very large amounts of PRL, compared with pituitaries from DES-treated rats, rat prolactin mRNA as evaluated by mean grain counts was considerably less in the MtT/W15 tumor than in DES-treated pituitary cells. These results show that in situ hybridization histochemistry can be used to detect changes in rat prolactin mRNA in tissue sections from the anterior pituitary with chronic estrogen treatment and that these pituitaries show a diffuse increase in immunoreactive prolactin cells and cellular prolactin mRNA, rather than distinct adenomatous areas within the glands. (Am J Pathol 1986, 125:35–44)

CHRONIC estrogen treatment produces pituitary prolactin cell hyperplasia and leads to the development of pituitary tumors in male and female rats.[1-12] These changes are associated with increased serum prolactin and pituitary prolactin (PRL) messenger RNA (mRNA).[13-19]

Although the hyperplastic pituitary gland tissues resulting from estrogen stimulation are commonly designated as tumors, the histology of these pituitaries is different from most pituitary tumors. For example, spontaneous tumors in aging rats exhibit an expanding mass of relatively homogeneous pituitary tissues with compression of adjacent normal anterior pituitary cells.[20] Our previous studies of pituitaries from diethylstilbestrol (DES)-treated rats revealed primarily diffuse hyperplasia of PRL cells with an intimate mixture of other anterior cell types associated with the hyperplastic PRL cells. Furth et al[21,22] showed that DES-treated pituitaries do become true neoplasms after several generations of transplantation, and these transplantable tumors (eg, the MtT/W15 tumor) have been studied by many investigators.[9,23] However, the transition between hyperplasia and the initial development of pituitary tumors is not clearly defined. Thus, immuno-histochemical studies that localize intracellular PRL production do not clearly outline tumors in DES-treated rats, even though the pituitary glands are 5–10 times larger than those seen in untreated rats.

The technique of in situ hybridization histochemistry is becoming widely used to detect specific mRNA species in tissues and in isolated cells.[24-30] This technique demonstrates the cellular localization of mRNA coding for specific proteins and can be used in visualizing the primary products of gene expression while preserving morphologic information. This technique demonstrates the cellular localization of mRNA coding for specific proteins and can be used in visualizing the primary products of gene expression while preserving morphologic information. This technique may also be used in the study of changes in gene regulation and cellular morphologic features during the development of neoplasms as well as in examination of the hormonal regulation of gene expression at the cellular level.[26]

The present report examines the localization of rat PRL mRNA in the pituitary during the development of DES-induced pituitary lesions and in pituitary neoplasms by *in situ* hybridization histochemistry. Our aim was to determine whether PRL mRNA was concentrated in specific areas of the pituitary or was diffusely localized in DES-induced hyperplastic and neoplastic pituitary tissues.

Materials and Methods

Animals

Forty-day-old female Wistar/Furth (W/Fu) rats were purchased from Harlan Sprague-Dawley (Madison, Wis). The animals were maintained on a schedule of 12 hours light and 12 hours darkness and fed *ad libitum*. Silastic tubing with 10 mg DES was prepared as previously reported.[6,7] Implants were placed subcutaneously under light ether anesthesia through a small incision in the back. Control animals received empty Silastic tubing. Animals were sacrificed by decapitation after 3, 6, and 9 weeks. The transplantable MtT/W15 pituitary tumor was obtained from Dr. Bogden at the Mason Research Institute (Worcester, Mass). These tumors have been maintained in our laboratory by subcutaneous transplantation into the right flank of 40-day-old W/Fu rats. Palpable tumors were usually detected 20–40 days after transplantation of a 2-cu mm portion of tissue.

Immunohistochemical Staining

Paraffin-embedded tissues were cut a 5–6 μ and stained by the avidin–biotin–peroxidase complex (ABC) method as previously described.[6,7] The antisera were provided by the National Institute of Arthritis, Diabetes, Digestive and Kidney Diseases (NIADDK). Rat anti-PRL produced in rabbits, rat anti-growth hormone produced in monkeys, and human anti-adrenocorticotropic (ACTH) hormone produced in rabbits were used at 1:1000 dilution. After inhibiting endogenous peroxidase with 1% methanol-H_2O_2, cells and tissues were washed in phosphate-buffered saline (PBS), then treated with normal goat serum for suppression of nonspecific binding for 30 minutes. The primary antisera were applied for a 1-hour incubation at 23 C. The tissues were then washed for 30 minutes with PBS treated with a biotinylated second antibody, followed by PBS washes, and then incubated by the ABC method (Vector, Burlingame, Calif) for 30 minutes. Diaminobenzidine (DAB) was used as the chromogen (20 mg DAB in 50 ml PBS, pH 7.2, and 0.001% H_2O_2) for 5 minutes.

Controls consisted of substituting normal rabbit serum for the primary antiserum and absorbing each antiserum with specific antigens for 24 hours before performing the assay.

Preparation of Double Stranded Complementary DNA for Rat PRL

A PBR 322 clone containing cDNA complimentary to rPRL mRNA was obtained from Dr. R. Maurer (University of Iowa). Recombinant DNA was isolated and purified by the method of Birnboin and Doly.[31] The 900 base pair (bp) insert was removed by Pst I digestion,[32] purified on a 4% sodium dodecyl sulfate (SDS)-polyacrylamide gel, and quantitated by fluorometry.[33] The cDNA insert was nick-translated with the use of a kit supplied by Bethesda Research Laboratories (Gathersburg, Md). The 50-μl reaction, containing 400 ng of cDNA and 1 nmol each of ^{3}H-dCTP and ^{3}H-TTP was incubated for 90 minutes at 15 C. Specific activities of the probes ranged from 0.5 to 1.0 × 10^7 cpm/μg.

After purification on a Sephadex G75 column, aliquots of the labeled probe were treated with 10 ng/μl DNase I (Amersham, Arlington Heights, IL) for 15 minutes at 37 C and then ethanol-precipitated. The precipitates were pelleted by centrifugation at 20,000g for 15 minutes at −20 C, then resuspended in Tris-EDTA buffer.

The purity and size of the ^{3}H-cDNA probes were examined with 4% SDS polyacrylamide gel electrophoresis. The conditions of DNase treatment were developed to produce probes consisting of 40–50 base pairs, judged by the migration of bromophenol blue, xylene cyanol, and appropriate standards.

In Situ Hybridization

The procedure of Hoshina et al was used with some modifications.[27] Pituitary tissues were rinsed in PBS for removal of blood and serum, cut into small pieces (5 × 5 × 5 cu mm), embedded in OCT compound (Lab Tek Products, Miles Laboratory, Inc., Naperville, IL), immersed in liquid nitrogen, and stored at −70 C. Rat liver tissue was frozen in a manner similar to that used for the pituitary.

Embedded tissue was trimmed into 4 × 4 mm squares and sectioned at 8 μ in a cryostat (American Optical Corp., Southbridge, Mass) at −20 C and placed 3 cm from the edge of a treated slide (see below). Two pieces of tissue were placed 5 cm apart on the same slide.

After air-drying for 10 minutes at room temperature, the sections were fixed in ethanol/glacial acetic acid (3:1) for 20 minutes at 4 C and in 2× standard saline citrate (SSC) (300 mM NaCl, 30 mM sodium citrate). After washing with 2X SSC, the slides were dehydrated by

immersion in 75% and 95% ethanol, air-dried, and stored at −70 C.

Microscopic slides were incubated for 3 hours at 70 C in a solution of 3× SSC (350 mM NaCl, 45 mM sodium citrate) (pH 7.0), 0.02% Ficoll, 0.02% polyvinylpyrrolidone, and 0.2% gelatin. They were then dipped in water and fixed for 20 minutes in ethanol/glacial acetic acid (3:1), then dried at 70 C in an oven overnight. To reduce nonspecific adsorption of the probe, glass coverslips (Fisher Scientific Co., Detroit, Mich) were immersed in Sigmacote (Sigma Chemical Co., St. Louis, Mo) for 2 seconds and dried in an oven at 70 C overnight.

Hybridization

The cut sections were treated with 0.2N HCl for 20 minutes at room temperature and rinsed in water for 5 minutes and for 30 minutes with 2× SSC at 70 C. After washing in water for 5 minutes, the slides were dehydrated in 75% and 95% ethanol and air-dried. The sections were treated with hybridization medium (600 mM NaCl, 10 mM Tris-HCl [pH 7.5], 1 mM EDTA, pH 7.5, 0.02% Ficoll 400, 0.02% polyvinylpyrrolidimine, 0.1% salmon sperm DNA, 0.1% yeast tRNA, 0.01% poly A and poly C, 50% formamide [deionized for 2 hours with 5g Bio Rad RG 501-X8 per 100 ml], and 10% dextran sulfate [Sigma]). Each section was treated with 20 μl of this medium and incubated in a moist chamber for 2 hours. After rinsing in water for 5 minutes, the sections were again dehydrated with 75% and 95% ethanol.

The cDNA probe was denatured by boiling for 10 minutes. Hybridization was performed about 4 ng of ³HcDNA (50,000 cpm) per 100 μl of medium, covered with a siliconized cover glass, in a moist chamber at room temperature for 18 hours. After the cover glass was removed, the slide was washed with 2× SSC:0.1% SDS for 4 hours, 1× SSC:0.1% SDS for 2 hours, and 0.5 × SSC:0.1% SDS for 1 hour.

Autoradiography

After dehydration in 75% and 95% ethanol, the slides were dipped in nuclear track emulsion (Kodak NTB-2) (Eastman Kodak, Rochester, NY), diluted with water and 0.3 M ammonium acetate (1:1), and prewarmed to 45 C.[35] Slides were dried in an upright position for 1.5 hours and exposed in a light-proof box for 2-3 weeks at 4 C. The slides were developed with Kodak D-19 developer (3 minutes at 20 C), rinsed in cold water for 1 minute, fixed with Kodafix (5 minutes at 20 C), washed for 2 hours, and then stained with hematoxylin and eosin (H&E).

Quantitation

Photomicrographs were taken at 400× from random fields and enlarged ×3. The number of grains over the tissue was counted, and the distribution of grains over random sections from pituitary tissues was counted. Mean grain count (MGC) was calculated with the following formula[36]:

$$\frac{\text{Number of grains}}{\text{number of nuclei} \times \text{weeks of exposure}}$$

In Situ Hybridization and Immunohistochemistry

In one experiment tissues were fixed in 4% buffered paraformaldehyde, pH 7.4, instead of acetic acid and ethanol. After overnight hybridization, the tissues were washed, then stained by immunohistochemistry as previously described with rat PRL antiserum. Washes between steps were done with 1× SSC instead of PBS. Autoradiography was performed after immunostaining as previously described.

Cell Count

The number of positively stained cells after immunohistochemistry was counted by systematically sampling

Table 1—Effects of DES Treatment on Pituitary Wet Weight, Serum PRL, and GH Cells Determined by Immunohistochemistry in Female Rats

Weeks of DES treatment	Pituitary weight (mg)	Serum PRL (ng/ml)	Immunoreactive cells (%)	
			PRL	GH
Control	7.9 ± 0.31	47.1 ± 8.9	34 ± 2	38 ± 1
3	18.1 ± 1.67*	49.1 ± 108*	40 ± 3	36 ± 1
6	28.4 ± 1.52*	1332 ± 238*	50 ± 7	26 ± 5†
9	51.6 ± 2.4*	4155 ± 571*	69 ± 3*	21 ± 2*
MtT/W15 tumor	7.6 ± 0.57	32000 ± 4200	66 ± 1	24 ± 1

† $p < 0.05$.
* $P < 0.001$, compared with control animals. The percentage of cells positive for PRL and for GH was derived as described in Materials and Methods. Values are mean ± SEM for n = 4.

multiple areas from the slide using a 4-sq mm grid in the microscope ocular. Cells were counted at ×400. A minimum of 1000 cells were counted and scored as positive or negative if a brown-black reaction produce (positive cells) was present in the cytoplasm.

Statistical evaluation was done with the Student t test.

Radioimmunoassay

Serum samples were collected at the time of sacrifice and frozen at −20 C. Radioimmunoassay for serum PRL was done as previously described.[7]

Results

Effects of DES

DES treatment for 3-, 6-, and 9-week periods produced an increase in pituitary gland weights, serum PRL levels, and an increase in the percentage of PRL-positive cells by immunostaining (Table 1). There was a twofold increase in PRL-positive cells after 9 weeks of DES treatment. Staining for GH and ACTH showed that these cells were intimately admixed with the hyperplastic cells, and distinct tumor masses were not seen.

Hybridization

The effects of probe size on the hybridization signal was evaluated by performing hybridization histochemistry with a large (900 bp) and a small probe (40–50 bp). The results shown in Table 2 indicated that the smaller probe produced a 3.5-fold increase in signal strength as compared with the 900 bp probe. The smaller probes were used in all subsequent experiments.

When sections of normal rat pituitaries were hybridized to nick-translated probes complementary to rPRL mRNA, the distribution of grains was present predominantly over the anterior pituitary (Figure 1). The posterior pituitary and intermediate lobes contained a number of grains that was equivalent to background and was significantly less than the heavily labeled anterior lobe (Figure 1). The mean grain count (MGC) increased

with increasing duration of DES treatment. After 9 weeks of DES treatment, there was a 2.3-fold increase in MGC, compared with control pituitaries. The distribution of grains in the enlarged pituitary glands was diffuse (Figures 2 and 3), and there were no distinct foci of heavily labeled cells. Serial sections of pituitary tissues stained for PRL also showed a diffuse distribution of PRL-producing cells in the enlarged anterior pituitary gland.

Specificity of Hybridization

Several control studies were done to access the tissue and nucleic acid specificity of the in situ hybridization. Sections of liver hybridized with the rPRL cDNA probe under identical conditions as the pituitary tissues showed only a few scattered grains after exposure for 3 weeks (Figure 4). Also, pituitary sections were treated with pancreatic RNase (100 μg/ml Sigma) in PBS or buffer for 1 hour at 37 C, and washed with 2× SSC for 1 hour at 4 C. The slides were then processed for hybridization. RNAse pretreatment markedly reduced the number of grains, compared with sections not treated with this enzyme (data not shown). In additional control studies anterior pituitaries and MfT/W15 tumor tissues were hybridized with a nick-translated pBR322 probe (specific activity 0.5–1 × 10^7 cpm/μg). The conditions of hybridization, probe size, and radioactivity were identical to those used in the hybridization with the rPRL cDNA probe. Only background labeling was seen when the pituitary or MfT/W15 was hybridized with this probe (Figure 5).

Transplantable Tumor Tissues

Hybridization histochemistry with tissue sections from the MfT/W15 transplantable tumor resulted in deposits of silver grains over the entire tumor (MGC = 3.5 ± 0.59) (Figure 6). Immunohistochemical staining with antisera against GH and PRL showed that 24%

Table 2—Effects of Probe Size on the Signal Strength After in situ Hybridization Determined by Mean Grain Counts From Sections Hybridized to rPRL Probes

Probe Size*	No. Nuclei	MGC†
900 bp	250 ± 15	0.84 ± 0.06
40-50 bp	232 ± 10	2.9 ± 4.2‡

* MGC, mean grain count; background MGC based on pBR was <0.80. Mean ± SEM for four separate experiments.

† Probe size expressed as number of base pairs of nucleotides derived from polyacrylamide gel electrophoresis.

‡ P < 0.001, compared with 900 bp probe.

Table 3—Effect of Chronic Diethylsilbestrol Treatment on Mean Grain Count in Sections in Rat Anterior Pituitary Tissues

Weeks of DES treatment	No. nuclei	MGC
Control pituitary	268 ± 12	2.8 ± 0.09
3	238 ± 32	4.9 ± 0.23*
6	257 ± 32	5.7 ± 0.44*
9	253 ± 25	6.5 ± 0.49*
MtT/W15 tumor	235 ± 13	3.5 ± 0.59

* P < 0.001, compared with control pituitary sections.

Background MGC based on pBR322 control was <0.80. Mean ± SEM for four separate experiments. Hybridization was done with a 40–50 bp PRL cDNA probe was described in Materials and Methods.

Figure 1—Demonstration of rat PRL mRNA in rat anterior pituitary tissue by *in situ* hybridization with anterior pituitary tissue from untreated controls. Heavy labeling is present over the anterior pituitary, whereas the intermediate lobe (*I*) shows only background labeling (Autoradiography and H&E, ×330). The **Inset** shows several cells that are heavily labeled, as well as unlabeled cells (*arrow*) at a higher magnification. (**Inset**, autoradiography and H&E, ×630)
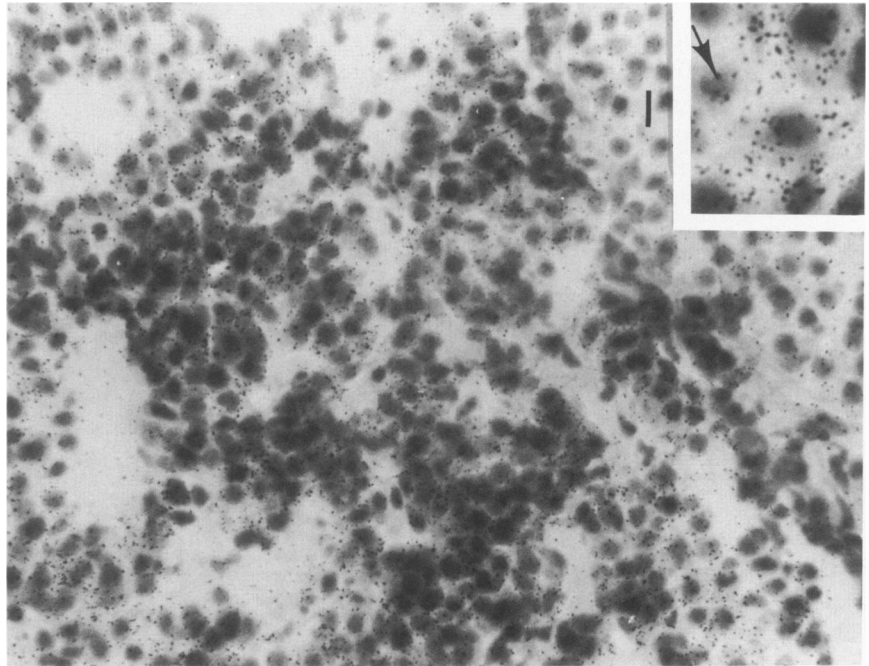
± 1% of the tumor cells were positive for GH and 66% ± 1% were positive for PRL. The adjacent areas of skeletal muscle in the tumor showed only a few deposits of silver grains, unlike the heavy labeling observed over the tumor cells.

The combined procedures of hybridization histochemistry followed by immunohistochemistry revealed deposits of silver grains over cells that were positive for PRL as well as in tumor cells that did not stain with the PRL antiserum (Figure 6, inset).

Discussion

The present study shows that chronic DES-treatment results in an increase in rPRL mRNA as determined by *in situ* hybridization histochemistry. With this tech-

Figure 2—*In situ* hybridization in rat anterior pituitary tissues after 3 weeks of DES treatment. There is an increase in the MGC in comparison with control anterior pituitaries. (Autoradiography and H&E, ×330)
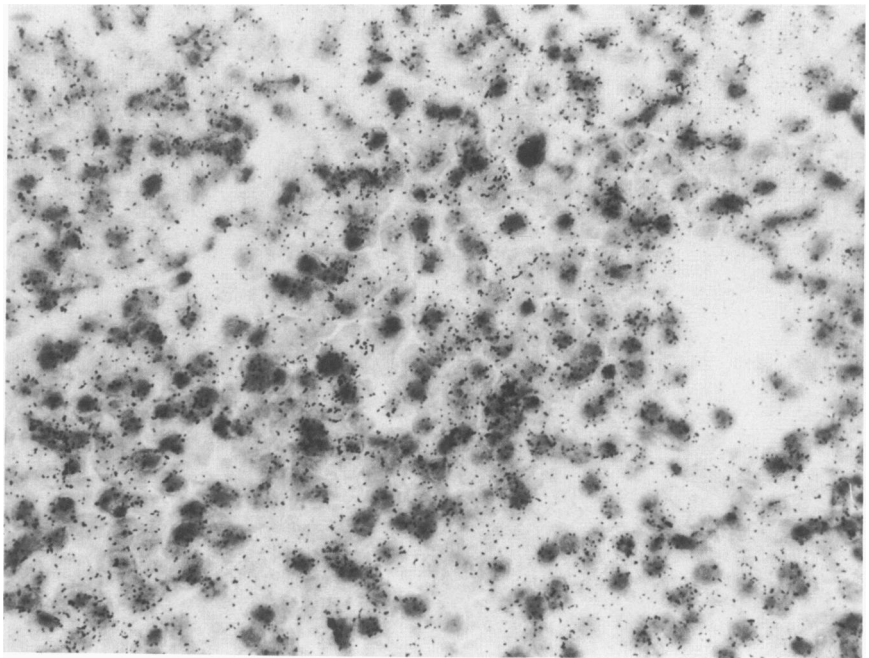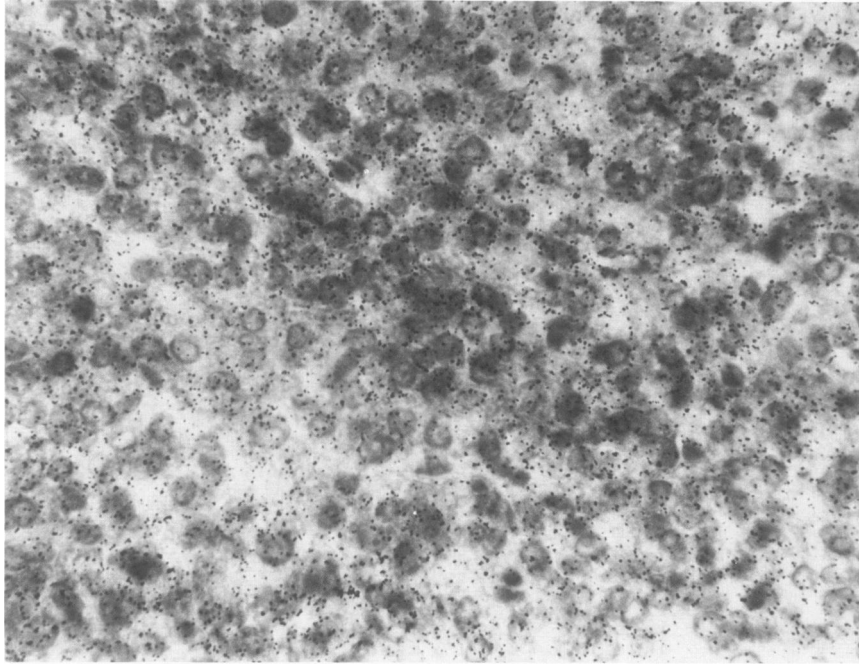
Figure 3—*In situ* hybridization in rat anterior pituitary after 9 weeks of DES treatment. The MGC is increased above that observed after 3 weeks of DES treatment. (Autoradiography and H&E, ×330)

nique, we showed that the increase in mRNA was distributed diffusely in the anterior pituitary gland and that there was not a focal or localized distribution of mRNA. This agrees with our immunohistochemical analysis of the distribution of PRL in the anterior pituitary. A previous study on the localization of prolactin mRNA on histologic sections by *in situ* hybridization showed radioautographic labeling over weakly acidophilic cells in the rat anterior pituitary gland.[37] These results are

also in agreement with the recent report of Nogami et al, who reported that the number of grains per cell was increased after estrogen treatment for 5 days and *in situ* hybridization studies with a ³H-labeled PRL cDNA probe.[38] Our results showing an increase in rPRL mRNA are also in agreement with other studies using solution hybridizations that showed a 4-fold increase in prolactin mRNA sequences in the pituitary after 2 weeks of estrogen treatment.[16] The increase in PRL
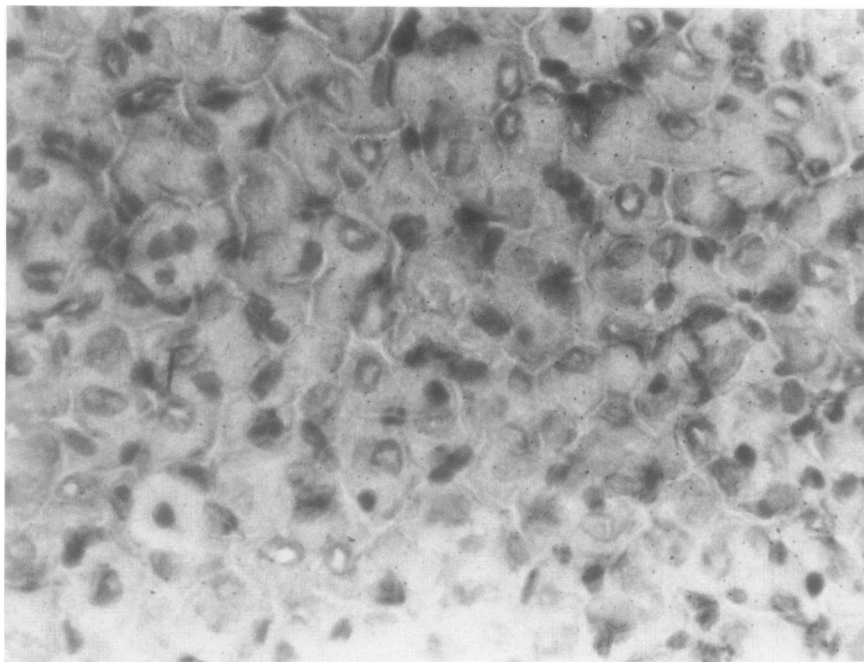
Figure 4—Sections of rat liver hybridized with rPRL cDNA (50,000 cpm) with probe and conditions identical to those used with normal and hyperplastic pituitaries. Labeling was equivalent to background with a mean grain count <0.80. (Autoradiography and H&E, ×330)

Figure 5—*In situ* hybridization of MtT/W15 tumor tissue with pBR322. This cDNA probe was prepared by nick-translation (specific activity 1×10^7 cpm/μg), this preparation was similar to preparation of the rPRL cDNA probe. The hybridization with 50,000 cpm was performed under the same conditions as the rPRL cDNA probe. (Autoradiography and H&E, ×300)
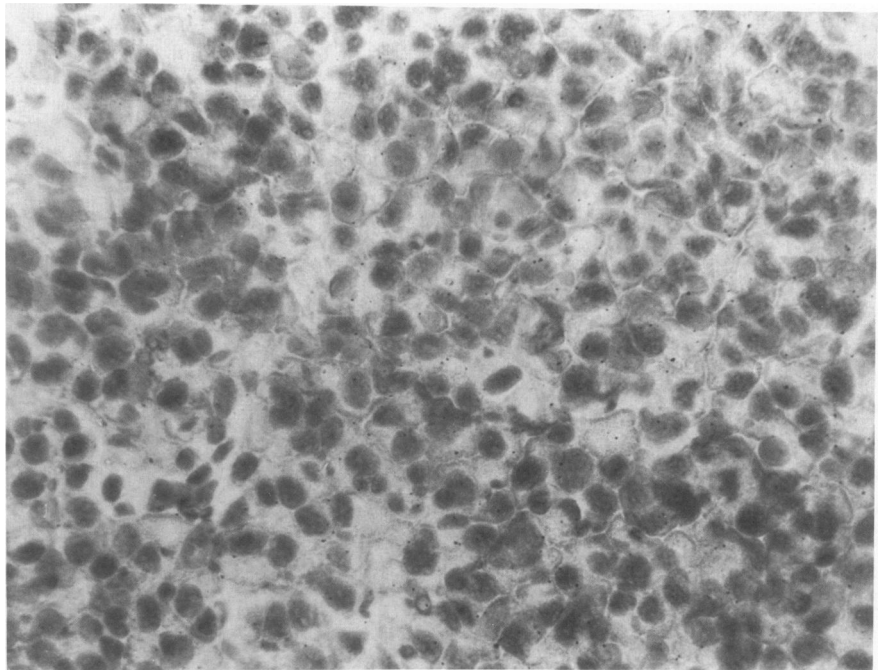
mRNA is also similar to increases in PRL synthesis[15] and translatable mRNA levels[19] induced by estrogen treatment under similar conditions. In the present study there was a 1.8-fold increase in MGC after 3 weeks of DES treatment and a 2.3-fold increase in the MGC after 9 weeks of DES treatment. Although there was an increase in the PRL-producing cells as well as in the MGC, the increase in MGC exceeded the increase that would have been expected from merely an increase in

PRL cells (ie, the MGC was expressed as grains per cell nuclei). Concomitantly, there were 1.2-fold and 2.0-fold increases in the percentage of PRL positive cells after 3 and 9 weeks of DES treatment, respectively.

The use of *in situ* hybridization provides another way of examining regulation in cells by studying the mRNA for specific proteins in addition to the proteins. Our present studies suggest that DES causes a marked increase in PRL production, but only a relatively smaller

Figure 6—*In situ* hybridization with MtT/W15 tumor tissues and rPRL cDNA. Labeling is present over most of the tumor cells. (Autoradiography and H&E, × 330). The inset shows the combined procedures of *in situ* hybridization followed by immunoperoxidase staining with PRL antiserum in a separate experiment. The dark brown cytoplasmic reaction product reveals stained PRL in some of the tumor cells (*arrows*). (Inset, autoradiography and immunoperoxidase, ×728)
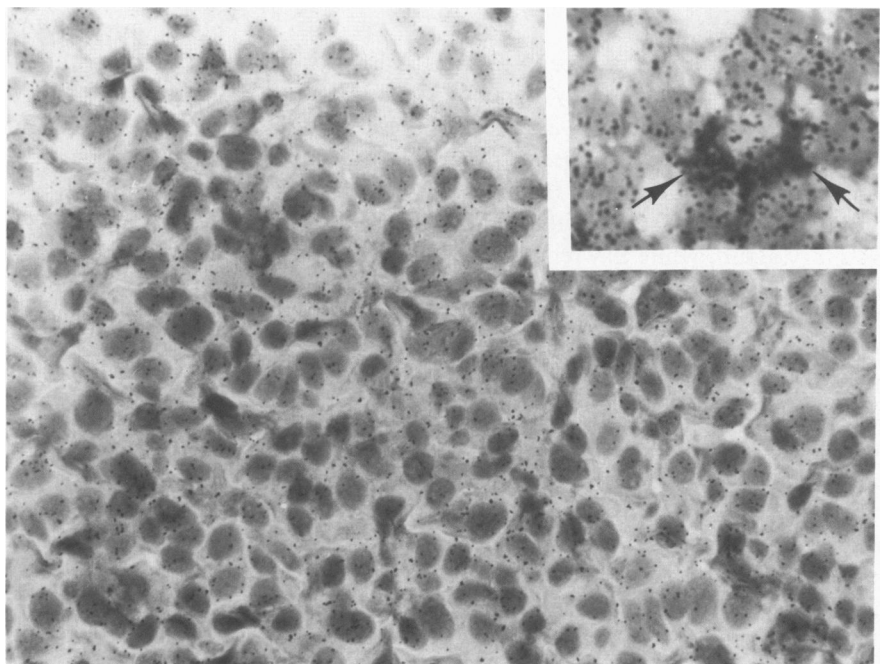
increase in PRL mRNA synthesis. These studies also suggest that although the MtT/W15 tumor is producing very large amounts of PRL, compared with DES-treated pituitaries, the relative amounts of mRNA are much less in the transplantable MtT/W15 tumor than in DES-treated rat pituitary cells.

Hybridization histochemistry offers some advantages over immunohistochemistry, including the ability to visualize the anatomic sites of gene expression and to separate *de novo* synthesis of an intracellular protein from uptake.[39] However, there is more variation between experiments with hybridization histochemistry using nick-translated probes than with immunohistochemistry. Although some authors have reported that the use of ^{32}P-labeled probes has resulted in the identification of individual cells, preliminary experiments with ^{32}P-labeled probes have been less effective in revealing individually labeled cells in our studies. The use of ^{3}H-labeled probes with smaller amounts of radioactivity and longer incubation periods of up to a few days have decreased background labeling in a recent study.[40]

In situ hybridization studies were helpful in visualizing the cellular localization of specific mRNA species. Our results showed that heavy labeling for PRL mRNA was found only in the anterior lobe of the pituitary and not in the intermediate or in the posterior pituitary lobes. This observation is important, because recent evidence with radiolabeled DNA complementary to rat pituitary prolactin mRNA analyzed by hybridization studies[41] and immunohistochemical studies[42] suggests that rat hypothalamic tissues synthesize and store PRL. The latter studies have shown that PRL-like immunoreactivity is localized in nerve terminals of the rat hypothalamus.[42]

The successful demonstration of mRNA by *in situ* hybridization histochemistry depends on numerous parameters which are quite variable.[24-30] For example, investigators have used probes of varying sizes to obtain successful hybridization. Hudson et al[28] used a large probe (800 bp) to demonstrate GH mRNA in the rat pituitary gland, while Lawrence et al[29] showed that a 250–450 bp probe was more effective than ones of 50–200 or 600–1000 base pairs in the study of actin gene expression in cultured skeletal muscle myoblasts. Most other workers have suggested that probes smaller than 100 bp are most effective in hybridization histochemistry in tissue sections.[26,29] Our results show that a 40–50 base pair probe produced a 3.4-fold increase in signal strength, compared with a 900 base pair probe of rat PRL cDNA. While most investigators agree that treatment of tissue sections with 0.2 N HCl and with 2× SSC at 70 C increases the signal strength by increasing probe penetration into tissue sections, the use of pretreatment with proteinase K or other enzymes is more

controversial.[24,26,29] In our study proteinase K pretreatment did not increase the signal strength and also resulted in marked alteration of the tissue morphology (data not shown). The effects of various fixatives have also been examined. Although most of our experiments were done with ethanol/acetic acid fixative, several studies indicate that paraformaldehyde fixation[29] is as effective or more effective than ethanol/acetic acid fixatives. Our attempts to perform immunohistochemical staining on sections fixed with ethanol/acetic acid after hybridization were unsuccessful, whereas paraformaldehyde-fixed tissues resulted in satisfactory labeling by immunostaining after *in situ* hybridization.

In situ hybridization with rPRL cDNA in sections of MtT/W15 tumor tissue demonstrated a diffuse pattern of labeling in the tumor cells. Immunohistochemical staining with this tumor showed that 66% ± 1% of the tumor cells were producing PRL, whereas a smaller percentage produced GH (24% ± 1%). The dual production of PRL and GH by a smaller percentage of tumor cells has also been observed,[43] indicating that mRNA for PRL production may be functional in most of the cells within this tumor.

The effects of chronic DES treatment on the apparent increase in rPRL mRNA after 9 weeks of treatment is an estimate of the accumulation of total mRNA within the cells and does not indicate the point of hormonal regulation in this final mRNA. The half-life of the cytoplasmic mRNA could be affected by DES treatment,[17] and there may be differences in the half-life of mRNA among normal, DES-treated, and transplantable pituitary tissues. In some systems estrogen administration has been shown to increase the stability of ovalbumin mRNA.[44] Estrogen may also increase the level of transcription with an increase in the RNA polymerase activity[45] and in chromatin binding sites.[46] Estrogens have been shown to act directly on the pituitary *in vitro* to increase PRL synthesis and PRL mRNA, and to produce a slight increase in PRL cell numbers.[13,14] Recent evidence has shown that estradiol treatment in male and immature female rats stimulated the rate of PRL gene transcription.[47,48] Interestingly, the evidence suggested that estradiol stimulates PRL gene transcription by two independent mechanisms. The first phase of PRL gene transcription was independent of protein synthesis, whereas the second phase was inhibited by cyclohexamide.[48,49]

The current experiments show that chronic DES treatment produces a diffuse increase in the mRNA of anterior pituitary cells, along with an increase in the pituitary weight and the numbers of PRL-producing cells. Further information about the changes in mRNA during the progression from hyperplasia to the development of neoplasms will require additional studies of

pituitary tissues that have become relatively autonomous of estrogen-stimulated growth.

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