Estrogen-Induced Hyperplasia and Neoplasia in the Rat Anterior Pituitary Gland

An Immunohistochemical Study

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Diethylstilbestrol (DES) treatment of weanling F344 female rats resulted in enlarged pituitary glands and diffuse pituitary prolactin (PRL) cell hyperplasia in all animals after 9 and 12 weeks of treatment. Serum PRL was significantly greater than in control rats ($P < 0.001$). Immunohistochemical studies showed that most of the pituitary gland cells consisted of PRL cells. Ultrastructural studies showed increased numbers of PRL cells with hyperplasia of the rough endoplasmic reticulum and decreased numbers of secretory granules. There was a decrease in the relative number of growth hormone (GH) and other cell types in the anterior pituitary. Pituitary tumors and normal pituitary glands were dissociated with trypsin and maintained in culture for 3 weeks. The numbers of PRL and GH cells decreased with time in both groups, and there was an increase in the number of fibroblasts. Staining of the culture cells with neuron-specific enolase showed that the anterior pituitary cells were positive for this enzyme, while the fibroblastic cells were negative. When dissociated pituitary cells were cultured in the presence of 10^{-9} M DES for 7 days, there was a 42% increase in the number of immunoreactive PRL cells. These results indicate that DES-treated rats provide an excellent model for study of the *in vivo* and *in vitro* regulation of pituitary hyperplasia and neoplasia. (Am J Pathol 1983, 113:198-206)

PROLONGED ADMINISTRATION of estrogen has been shown to cause prolactin (PRL) cell hyperplasia and tumors in male and female rats.[1-10] Spontaneous development of pituitary tumors and pituitary hyperplasia is also common in some strains of aging rats.[11-15] A major problem with the use of spontaneous pituitary tumor development in aging rats as an animal model for the study of pituitary neoplasms is that the percentage of tumors and the time course of tumor development are difficult to control. Thus the use of a model that results in hyperplasia and tumors in animals within a relatively short period provides an experimental model that is more reproducible. This has been achieved in certain strains of rats by administration of estrogens.[4-9] Most of the lesions that develop after estrogen treatment have been called tumors, and a distinction between PRL cell hyperplasia and tumors has not been carefully examined except for a few reports in aging rats with spontaneous pituitary lesions.[13,15]

Although the mitogenic effects of estrogens on pituitary cells *in vivo* are well known, the effects of estrogen on pituitary cells in cultures are not well characterized. Previous investigators reported that estrogen did not cause growth stimulation *in vitro*,[16,17] but a recent report indicated that estrogen treatment *in vitro* produced PRL cell proliferation with an increased number of PRL cells.[18] Studies of pituitary cells in cultures are usually done with short-term cultures,[19,20,21] and there are few reports of pituitary cell cultures maintained for more than 2 weeks.[22,23] The development of an *in vitro* model for long-term culture of pituitary cells can be used for examination of the chronic effects of various secretagogues and other substances on pituitary cell proliferation and secretion.

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The purpose of this study was to examine the development of diethylstilbestrol (DES)-induced hyperplasia and neoplasia in the pituitary of young rats and to analyze immunohistochemically PRL and growth hormone (GH) cells maintained in culture for 3 weeks.

Materials and Methods

Materials

Thirty-day-old female Fischer 344 rats were purchased from Charles River Laboratories (Boston, Mass). The animals were maintained on a schedule of 12 hours of light and 12 hours of darkness and fed *ad libitum*. The method of Wiklund et al[8] was used to develop pituitary lesions with some modifications. DES was obtained from Sigma Chemical Co. (St. Louis, Mo). We used Dow-Corning Silastic tubing and silicone type A medical adhesive. Trypsin was from Difco Laboratories (Detroit, Mich) and Eagle's medium was from GIBCO (Grand Island, NY).

DES implants were prepared by cutting a 3-cm length of Silastic tubing (inside diameter 0.078 inches, outside diameter 0.125 inches), plugging one end with Silastic medical adhesive, and allowing it to dry overnight at room temperature. Then 10 mg of DES dissolved in 100% ethanol was added and allowed to evaporate overnight at room temperature. The open end was plugged with Silastic adhesive, and the implant was pretreated in 1% bovine serum albumin (BSA) overnight then used for implanting the following day. The implants were placed subcutaneously into 30-day-old female rats under light ether anesthesia through a small incision in the back. Control animals received empty pieces of Silastic tubing. The animals were sacrificed with ether anesthesia after 3, 9, and 12 weeks. Serum was collected and frozen for PRL assays and pituitary, uterus, adrenal, and total body weights were recorded and analyzed.

Cell Dissociation and Culture

The method of cell dissociation with trypsin was done as previously reported.[19] Briefly, the anterior lobes of pituitary glands were placed in sterile Eagle's medium (EMEM) with 1% penicillin, streptomycin, and 25 μg/ml fungizone and minced into small pieces with a sterile scalpel blade. The tissue was placed in a beaker with 0.25% (wt/vol) trypsin in EMEM for 45 minutes at 37 C. The tissues were dissociated by gentle suction with a Pasteur pipette, then centrifuged at 200*g* for 10 minutes at room temperature. The dissociated cells were counted in a hemocytometer, and viability was checked with 0.5% trypan blue solution.

Cells were cultured in EMEM with antibiotics, 15% horse serum, and 2.5% fetal calf serum. Three milliliters of cell culture medium was used in Costar flasks over 5% CO_2 at 37 C. The mediums were changed every 3 days. In some experiments aliquots of cells were cultured on glass coverslips in tissue culture dishes 35 mm in diameter. These cells were used for immunohistochemical stains, which were done directly on the coverslips. Six anterior pituitaries from 60-day-old F344 rats were dissociated with trypsin and cultured in EMEM for 7 days. Each culture dish received 5×10^5 cells. Serums were charcoal-treated to reduce the concentration of endogenous steroids.[24] DES was dissolved in ethanol and diluted in the medium for a final ethanol concentration of 0.1%. Cells were stained for PRL and GH after 3, 5, and 7 days in culture. A minimum of 200 cells per culture dish was counted as described below. The numbers of immunoreactive cells in the DES-treated cultures were expressed as the percentage of control cultures. Control cultures were treated with 0.1% ethanol only.

Immunohistochemical Staining

The cells were fixed in formalin for 1 hour or overnight at 23 C, then stained by the avidin-biotin peroxidase method, as previously described.[25] The antiserums used were provided by the National Institute of Arthritis, Metabolic, and Digestive Diseases (NIAMDD). Rat anti-PRL produced in rabbits, rat anti-GH produced in monkeys, and human anti-ACTH produced in rabbits were used at 1:1000 dilution. Rabbit anti-rat neuron-specific enolase (NSE) used at 1:1000 dilution was a gift from Dr. Paul Marangos (National Institute of Mental Health). 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 to suppress nonspecific binding for 30 minutes. The primary antiserums were applied for 1 hour or overnight incubation at 23 C. An additional incubation step with rabbit anti-human immunoglobulin (1:100) was used with the GH antibody for 30 minutes. Then the tissues were washed with PBS treated with 1:200 dilution of rabbit biotin-IgG made in goat for 30 minutes, followed by PBS washes, then incubation with avidin-biotin peroxidase complex (Vector, Burlingame, Calif) for 30 minutes. Diaminobenzidine (DAB) was used as the chromogen (10 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 antibody and absorbing each antibody with specific antigens for 24 hours before

Table 1—Determination of Pituitary and Uterine Weights and Serum Prolactin in Diethylstilbestrol-Treated and Control Rats

Weeks of treatment	Pituitary (mg)*		Uterus (mg)*		Serum prolactin (ng/ml)†	
	DES	Control	DES	Control	DES	Control
3	12.5 ± 0.5	5.3 ± 0.27	209 ± 10	118 ± 11	288 ± 66	75 ± 9
9	53.5 ± 2.6‡	9.5 ± 0.37	350 ± 16	181 ± 13	1250 ± 213‖	82 ± 8
12	163 ± 3.8§	12.7 ± 0.23	460 ± 18	260 ± 13	8884 ± 166‖	107 ± 9

* Mean ± SEM for 4 animals.
† Mean ± SEM for 3 animals.
‡ $P < 0.05$, compared with control.
§ $P < 0.01$, compared with control.
‖ $P < 0.001$, compared with control.

performing the assay. Additional controls consisted of omitting biotin-IgG or avidin-biotin peroxidase complex. All of these controls resulted in no staining of the tissues.

Prolactin Assays

Radioimmunoassays (RIAs) were done by the method and protocol supplied by the NIAMDD rat pituitary distribution program.

Cell Count

The number of positively stained cells after immunohistochemistry was counted by systematically sampling multiple areas from the slide using a 4-sq mm grid in the microscope ocular. Cells were counted at ×100 and ×400. A minimum of 200 cells was counted and scored as negative or positive if a brown-black reaction product was present in the cytoplasm.

Electron Microscopy

Small pieces of tissues, 1-3 cu mm, were fixed in 3% gluteraldehyde in phosphate buffer then postfixed in 2% osmium tetroxide. After staining with 2% urayl acetate, sections were embedded in Polybed/Araldite. Ultrathin sections were cut on a Reichert ultramicrotome, then stained with lead citrate and examined with a Zeiss 109 microscope.

Statistical Analysis

Statistical analysis was done with the Student t test. A P value ≤ 0.05 was statistically significant.

Results

There was a significant increase in pituitary weights after 9 weeks of treatment with DES implants (Table 1). Twelve weeks after implantation the pituitaries of DES-treated animals were 10–16 times heavier than those of control animals. After 9 weeks of DES treatment, about 20% of the pituitaries were hemorrhagic, and this increased to 80% after 12 weeks of DES treatment. DES treatment also resulted in a significant increase in uterine weights and a decrease in the total body weight of the animals. The mean body weight of DES-treated animals was 119 ± 2 g and that of controls was 163 ± 3 g (mean ± SEM) after 12 weeks.

The number of PRL cells was increased after 12 weeks of DES treatment. There was an increase in total number of pituitary cells in control animals also, because of the increase in pituitary weight with age (Table 2). However, the increase was seven times greater in DES-treated rats (Table 2). Immunostaining revealed an increase in the number of PRL cells (Figures 1-3).

Histologic examination of the pituitary glands showed hypertrophied cells with large nuclei and prominent nucleoli. Numerous mitotic figures were present. Immunostaining for GH, PRL, and adrenocorticotropic hormone (ACTH) revealed sheets of PRL cells, but GH- and ACTH-positive cells were admixed with the hypertrophied and hyperplastic PRL cells (Figure 4).

Ultrastructural examination showed many proliferating PRL cells identified by their characteristic pleomorphic granule morphology and large sizes (200-800 nm). The PRL cells contained abundant

Table 2—The Distribution of Number of Pituitary Cells in Diethylstilbestrol-Treated and Control Rats After Trypsin Treatment and Cell Dissociation

Weeks of treatment	n	No. cells/pituitary	
		DES	Control
3	6	0.98×10^6	0.65×10^6
9	6	2.0×10^6	1.1×10^6
12	6	16.0×10^6	2.4×10^6

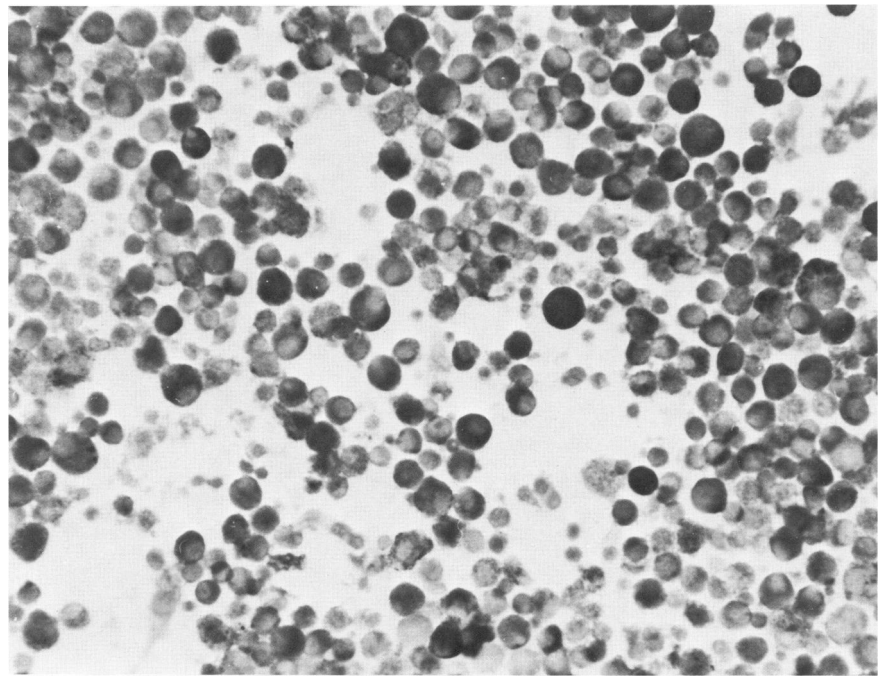


Figure 1 — Immunostaining of dissociated cells with PRL antiserum after 12 weeks of DES treatment. Many darkly staining PRL cells are present. (Immunoperoxidase, ×330)

rough endoplasmic reticulum and a moderate number of secretory granules (Figure 5). Active granule secretion by exocytosis was present in some cells. Other cell types were also identified by their characteristic morphologic features and granule sizes.[19] Prominent rough endoplasmic reticulum was noted in the gonadotropic cells that were identified by electron microscopy.

Serum PRL was significantly increased in DES-treated animals 3, 9, and 12 weeks after implantation (Table 1).

Approximately 70–80% of the seeded cells adhered to the culture flask. Cell viability was 90–95% before seeding. The cells assumed a spindled configuration after 3 days in culture (Figures 6 and 7) and could be removed after treatment with 0.1% trypsin for 5 minutes. The percentage of GH and PRL cells in culture were analyzed by immunostaining. Both cell types decreased with time in culture (Figure 8). However, after 3 weeks of DES treatment followed by cell dissociation and culture, the number of PRL cells from DES-treated rats increased at Day 8 in culture then subsequently declined (Figure 8). This increase was not seen with the 9- or 12-week DES-treated rat pituitary cells. After 21 days in culture immunoreactive PRL and GH cells were still present. There was a gradual increase in the number of fibroblasts, but these cells had larger nuclei and were negative after PRL and GH staining. After staining the cell cultures for NSE, the epithelial cells were positive and the fibroblastic cells were negative (Figure 7). Staining of the culture cells with NSE was used for estimation of the total number of epithelial cells. After 7 days of exposure to DES in culture, the total number of cells with PRL immunoreactivity was 42% greater than in control cultures. The cells with GH immunoreactivity were not increased in DES-treated cultures, compared with controls (Figure 9).

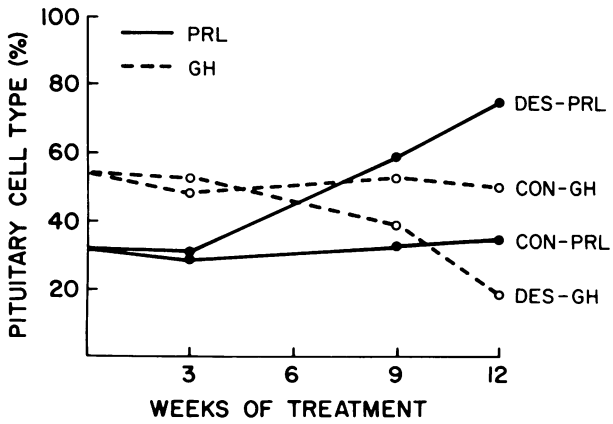


Figure 2 — Chronic DES treatment of F344 rats. There is an increase in PRL cells and a decrease in GH cells in DES-treated animals, compared with control (*CON*) rats.

Discussion

DES treatment of weanling female rats resulted in a 100% incidence of pituitary gland enlargement with a predominance of PRL cells. The increase in pitui-

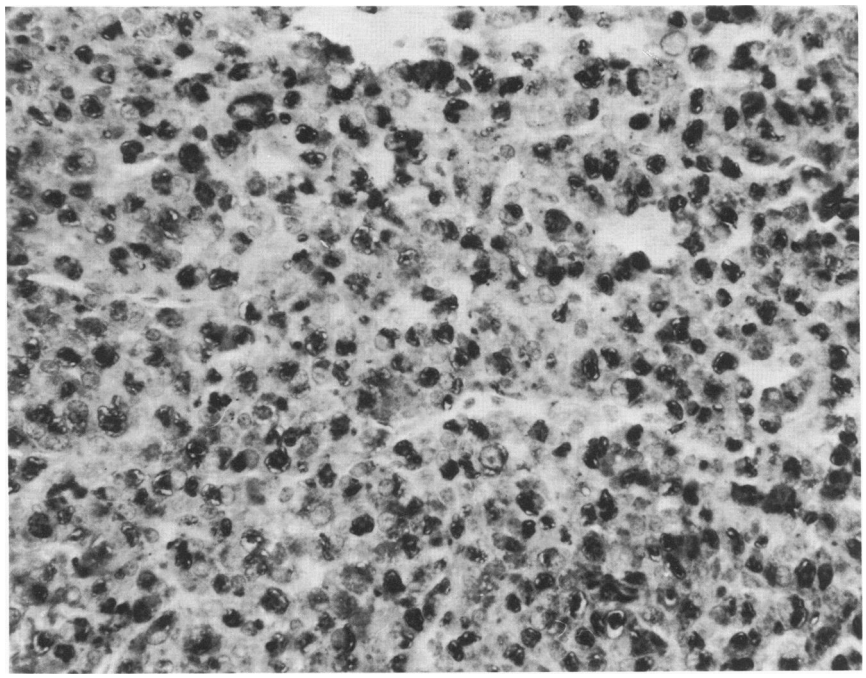


Figure 3—Immunostaining with PRL antibody in pituitary after DES treatment for 12 weeks. PRL-positive cells accounted for approximately 75% of the total pituitary cells. (Immunoperoxidase, ×330)

tary gland weight and PRL cell numbers was dependent on the time course of DES treatment. DES may be acting directly on the pituitary gland. Several investigators have shown that the pituitary gland and pituitary tumors contain estrogen receptors.[26,27] The uterus, which is another estrogen-sensitive tissue, also showed a positive response to DES stimulation, with a gradual increase in weight over the 12-week period in these experiments. The body weights of animals treated with DES decreased significantly over those of control animals. This finding agrees with a previous report on estrogen-induced pituitary tumors.[7] Gross examination of the mammary glands showed only a slight increase in size in the DES-treated animals. However, the mammary glands were not rated for development. Although DES can induce mammary tumors as well as pituitary and other tumors, the treatment periods in these experiments were probably too short for mammary tumor development. The use of weanling female rats provides a better model for use in obtaining larger tumors in a shorter time period. The reason for this difference may be related to an increased sensitivity of younger animals to DES or to strain differences. Previous reports have shown that Holtzman rats are relatively insensitive to DES-induced tumor development,[8] while F344, Wistar-Furth, and ACI strains are more susceptible to tumor development after estrogen treatment.[5-8] These differences have been shown to be inherited: crosses between DES-sensitive F344 and DES-resistant Holtzman rats have resulted in F1 hybrids with an intermediate level of susceptibility to DES-induced tumor development.[28] Recent evidence indicates that this genetic difference is due to a lack of a proliferation control mechanism in the F344 strains that functions in the Holtzman strain to turn off estrogen-stimulated pituitary cell proliferation.[9]

The histologic differences between the pituitaries of control animals and DES-treated rats were marked. Mitotic activity was a common finding, and this was rarely seen in the normal pituitaries. Areas of hemorrhage and focal necrosis due to the large sizes of the pituitaries were also present in DES-treated animals. Increased mitotic activity in pituitary cells after DES treatment in this report agrees with previous findings.[4,29] The hypertrophy and hyperplasia of PRL cells was diffuse in the gland, and discrete areas of multiple nodules or adenomas with a single cell type and compression of adjacent normal pituitary tissue were not present. This process of pituitary enlargement has been shown to be reversible after withdrawal of DES[8] and may be somewhat analogous to pituitary enlargement occurring during pregnancy. When these stimulated glands are transplanted under the renal capsule, cell proliferation continues in the presence of estrogen.[3,8] When the

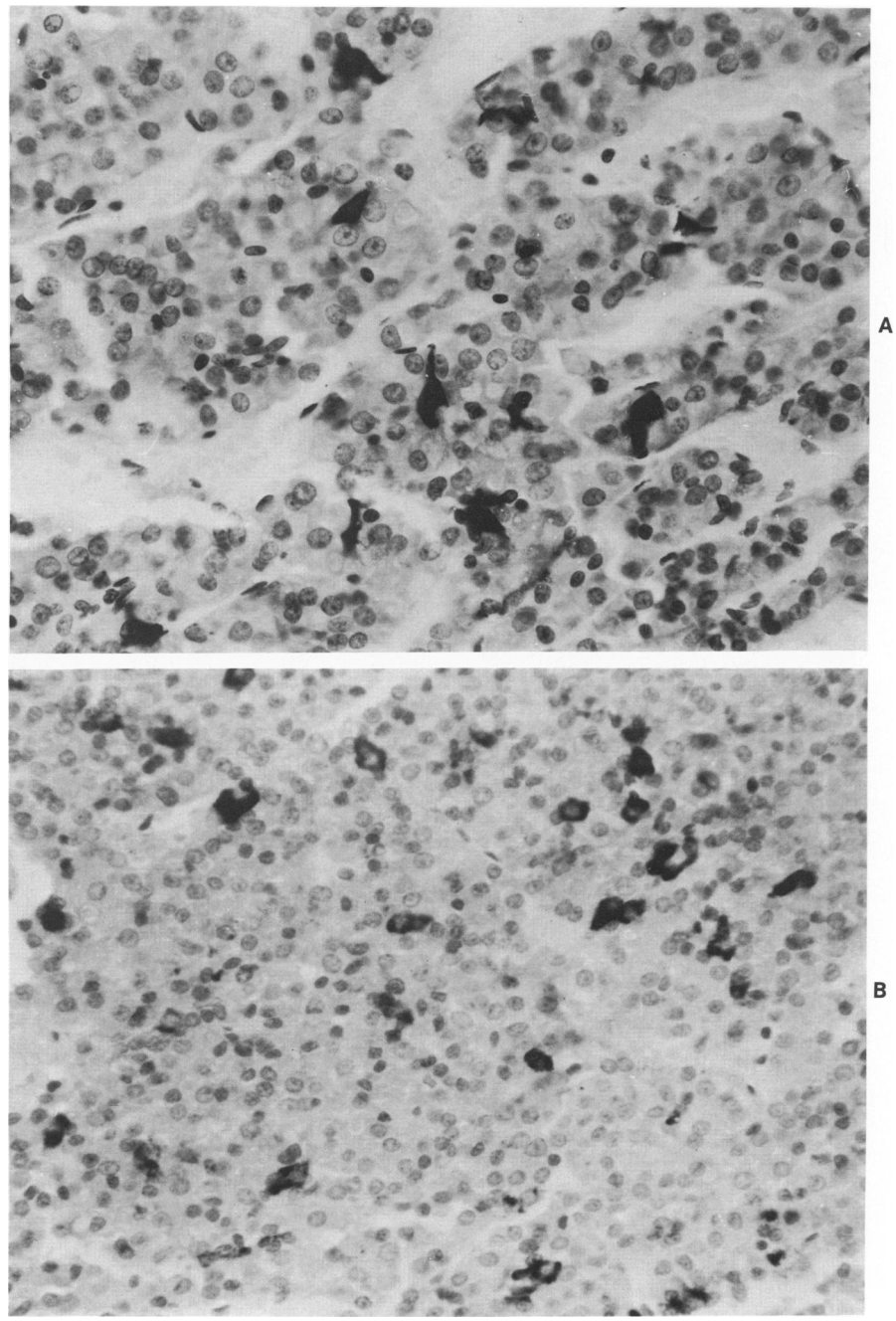


Figure 4A – Immunostaining with ACTH antibody in the anterior pituitary after DES treatment for 12 weeks. A few darkly stained ACTH cells are present among the predominant population of PRL cells, which are unstained. (Immunoperoxidase, ×330) **B** – Immunostaining of the same pituitary tissues for GH shows a small percentage of GH-positive cells, while most of the cell population which are PRL cells are unstained. (Immunoperoxidase, ×330)

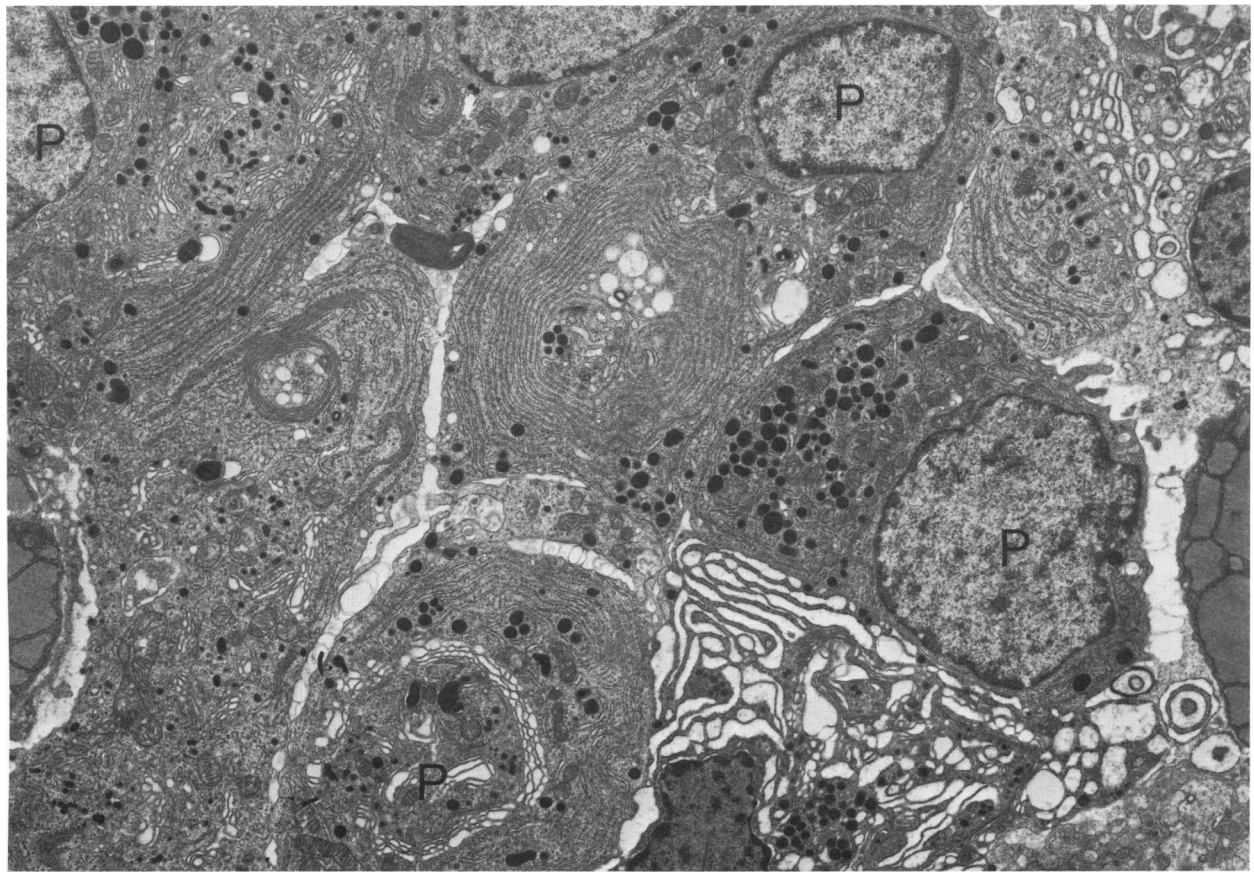


Figure 5—Electron micrograph of DES-treated rat pituitary cells. Many PRL (*P*) cells with abundant rough endoplasmic reticulum and pleomorphic dense core granules are present. (×5000)

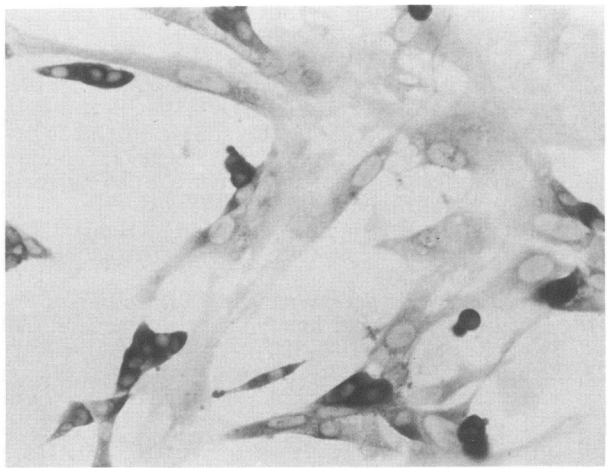
pituitary glands cease being merely hyperplastic and become true neoplasms is not well known. However, the use of this model can aid in answering this question.

The ultrastructural changes of marked cellular hypertrophy with increased protein-synthetic machinery, including abundant rough endoplasmic reticulum and the presence of reverse exocytosis, are similar to those of PRL tumors seen in rodent and human PRL adenomas.[13,25]

Immunohistochemical stains were the most helpful methods of establishing the type of hormones that various cell types produced. The specificity and sensitivity of this procedure allowed for a quantitative assessment of the various cell types in the dissociated cells. The avidin-biotin peroxidase procedure has been shown to be more sensitive than the Sternberger procedure and is probably the method of choice in detecting small amounts of intracellular hormones in the fixed tissues.[30-32]

The studies of DES-treated cells maintained in culture for up to 3 weeks are the first reported experiments of this kind. The results indicate that pituitary cells from control as well as DES-treated animals can be maintained for several weeks in a defined media and that some cells retain cytoplasmic PRL and GH. The increase in PRL-positive cells noted after 8 days in culture in the DES-treated animals suggests that the *in vivo* effects of DES in stimulating cell proliferation may have continued for a while *in vitro*. Some recent evidence suggests that estrogens may cause PRL cell proliferation *in vitro* as well.[18] The high plating efficiency and viability of the cultured cells indicates that an *in vitro* system of examining DES treatment is feasible with the methods used in these experiments. The culture system used in these experiments maintained viable pituitary cells with immunoreactive PRL and GH for 21 days in spite of the proliferation of fibroblasts. This is the first report to show that NSE can be used to distinguish between anterior pituitary cells that stain positively and fibroblastic cells which are negative for this marker. NSE has been found in normal human pituitary cells, pituitary tumors, and many cells of the diffuse neuroendocrine system.[33,34]

When the pituitary cells were treated with DES for



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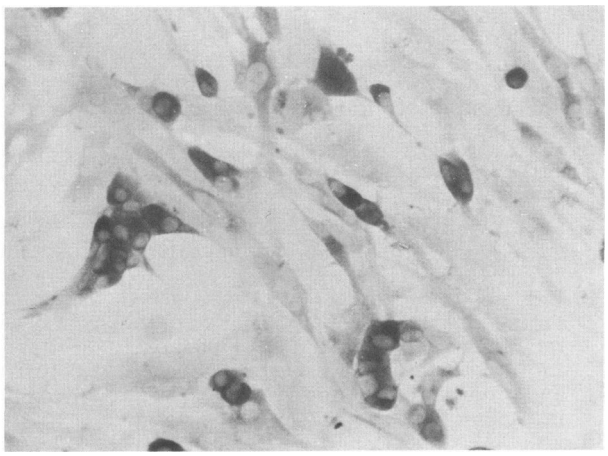

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Figure 6—Immunostaining with PRL antibody of dissociated pituitary cells from rats treated with DES for 9 weeks. After 8 days of culture many darkly stained PRL cells are present. Unstained fibroblasts with larger nuclei are also present. (Immunoperoxidase, ×330) **Figure 7**—Immunostaining with neuron-specific enolase antibody of trypsin-dissociated pituitary cells from rats treated with DES for 9 weeks. Neuron-specific enolase stains all of the anterior pituitary cells positively, but the fibroblastic cells are negative after 8 days of culture. (Immunoperoxidase, ×330)

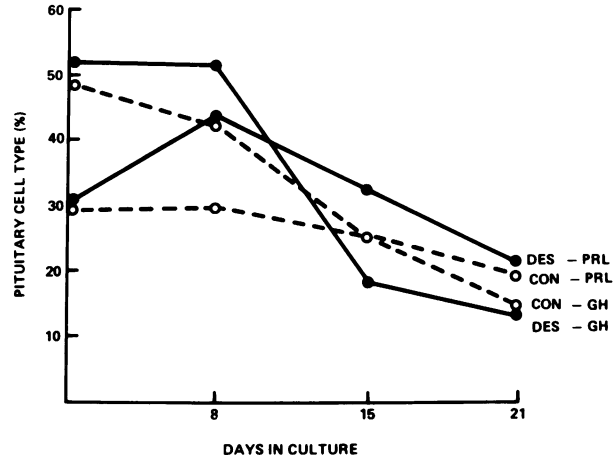

Figure 8—Distribution of PRL and GH immunoreactive cells in culture. Some animals were treated with DES for 3 weeks. Cells were maintained for 21 days in EMEM over 5% CO_2, 95% air. Immunostaining was done on coverslips.

7 days, there was an increase in the number of immunoreactive PRL cells. This finding suggests that DES has a direct effect on anterior pituitary cells *in vitro*. Other workers have reported an increase in pituitary PRL cells *in vitro* after incubation in the presence of estradiol.[10] Lieberman et al also showed an increase in prolactin mRNA levels in cells that were cultured in the presence of estradiol.[10]

PRL pituitary adenomas are the most common type of pituitary tumors in humans.[35] Although estrogens have a key role in the development of these lesions in the rat pituitary, the role of hormones in the development of PRL adenomas in humans is not clear.[36] Since most PRL-producing adenomas occur in premenopausal women, the rat model of DES-induced tumors in young rats may be an excellent model for the study of the mechanism of pituitary tumor development. Although various models of spontaneous tumor development are available in aged rats, the time of tumor development and the incidence of tumors are quite variable.[13-15]

With the use of the DES-induced hyperplastic and neoplastic pituitaries in young rats, various morphologic, biochemical, and hormonal studies can be done that may provide clues to the etiology and biology of human prolactinomas.

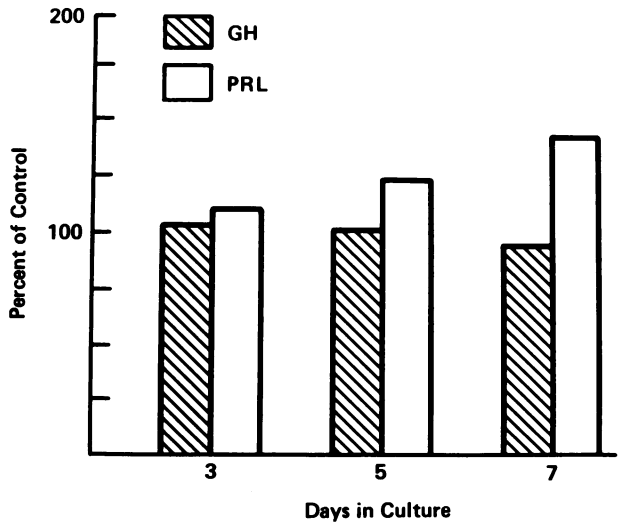

Figure 9—Effect of DES on PRL and GH cells in culture. Cells were incubated with 10^{-9} M DES for 7 days. The numbers of PRL and GH cells were identified by immunohistochemistry and compared with control cultures, which were incubated without DES. Each point represents the mean of two culture dishes.

References

1. Furth J, Ueda G, Clifton KH: The pathophysiology of pituitaries and their tumors: Methodological advances, Methods in Cancer Research. Vol 10. Edited by H Bush. New York, Academic Press, 1973, pp 201-277
2. Furth J, Nakane P, Pasteels JL: Tumors of the pituitary gland, Pathology of Tumors in Laboratory Animals. Vol 1. Edited by US Turosov. Lyon, France, IARC Scientific Publication, 1976, pp 201-237
3. Clifton KH, Meyer RK: Mechanisms of anterior pituitary tumor induction by estrogen. Anat Rec 1956, 125: 65-81
4. De Nicola AF, Von Lawzewifsch I, Kaplan SE, Libertun C: Biochemical and ultrastructural studies on estrogen-induced pituitary tumors in F344 rats. J Natl Cancer Inst 1978, 61:763-763
5. Holtzman S, Stone JP, Shellabarger CJ: Influence of diethylstilbestrol treatment on prolactin cells of female ACI and Sprague-Dawley rats. Cancer Res 1979, 39: 779-784
6. Stone JP, Holtzman S, Shellabarger CJ: Neoplastic response and correlated plasma prolactin levels in diethylstilbestrol-treated ACI and Sprague-Dawley rats. Cancer Res 1979, 39:773-778
7. Gersten BE, Baker BL: Long action of intrahyophyseal implants of estrogen as revealed by staining with peroxidase-labeled antibody. Am J Anat 1970 128:1-20
8. Wiklund J, Wertz N, Gorski J: A comparison of estrogen effects on uterine and pituitary growth and prolactin synthesis in F344 and Holtzman rats. Endocrinol 1981, 109:1700-1707
9. Wiklund JA, Gorski J: Genetic differences in estrogen-induced deoxyribonucleic acid synthesis in the rat pituitary: Correlation with pituitary tumor susceptibility. Endocrinol 1982, 111:1140-1149
10. Lieberman ME, Maurer RA, Claude P, Wiklund J, Wertz N, Gorski J: Regulation of pituitary growth and prolactin gene expression by estrogen, Hormones and Cancer. Edited by WW Leavitt. New York, Plenum Press, 1980, pp 151-163
11. Wolfe JM, Bryan WR, Wright AW: Histologic observation on the anterior pituitaries of old rats with particular reference to the spontaneous appearance of adenomata. Am J Cancer 1938, 34:353-372
12. Thompson SW, Hunt RD: Spontaneous tumors in the Sprague-Dawley rat: Incidence rates of some types of neoplasms as determined by serial sections versus single section technics. Ann NY Acad Sci 1963, 108:833-845
13. Kovacs K, Ilse G, Ryan N, McComb DJ, Horvath E, Chen HJ, Walfish PG: Pituitary prolactin cell hyperplasia. Hormone Res 1980, 12:87-95
14. Trouillas J, Girod C, Claustrat B, Cure M, Dubois MD: Spontaneous pituitary tumors in the Wistar/Furth/Ico rat strain: An animal model of human prolactin adenoma. Am J Pathol 1982, 109:57-70
15. Lee AK, DeLellis RA, Blount M, Nunnemacher G, Wolfe HJ: Pituitary proliferative lesions in aging male Long-Evans rats: A model of mixed multiple endocrine neoplasia syndrome. Lab Invest 1982, 47:595-602
16. Sorrentino JM, Kirkland WL, Sirbasku DA: Control of cell growth: I. Estrogen-dependent growth *in vivo* of a rat pituitary tumor cell line. J Natl Cancer Inst 1976, 56:1149-1154
17. Kirkland WL, Sorrentino JM, Sirbasku DA: Control of cell growth: III. Direct mitogenic effect of thyroid hormones on an estrogen dependent rat pituitary tumor cell. J Natl Cancer Inst 1976, 56:1155-1158
18. Lieberman ME, Maurer RA, Claude P, Gorski J: Prolactin synthesis in primary cultures of pituitary cells: regulation by estradiol. Mol Cell Endocrinol 1982, 25: 277-294
19. Lloyd RV, McShan WH: Study of rat anterior pituitary cells separated by velocity sedimentation at unit gravity. Endocrinology 1973, 92:1639-1651
20. Nakano H, Fawcett CP, McCann SM: Enzymatic dissociation and short term culture of isolated rat anterior pituitary cells for studies on the control of hormone secretion. Endocrinology 1976, 98:278-288
21. Lloyd RV, McShan WH: Effects of LH-RH on isolated pituitary gonadotropic cells. Proc Soc Exp Biol Med 1976, 151:160-162
22. Snyder J, Hymer WC, Wilfinger WW: Culture of human pituitary prolactin and growth hormone cells. Cell Tissue Res 1978, 91:379-388
23. Bethea CL, Weiner RI: Human prolactin secreting adenoma cells maintained on extracellular matrix. Endocrinology 1981, 108:357-360
24. Horwitz KB, Costlow ME, McGuire WL: MCF-7: A human breast cancer cell line with estrogen, androgen, progesterone and glucocorticoid receptors. Steroid 1975, 26:785-795
25. Lloyd RV, Gikas PW, Chandler WF: Prolactin and growth hormone producing pituitary adenomas: An immunohistochemical and ultrastructural study. Am J Surg Pathol 1983, 7:251-260
26. Leavitt WW, Kimmel GL, Friend JP: Steroid hormone uptake by anterior pituitary cell suspensions. Endocrinology 1973, 92:94-103
27. Sota AM, Sonnenschein C: Estrogen receptor levels in estrogen sensitive cells in culture. J Steroid Biochem 1979, 11:1185-1196
28. Wiklund J, Rutledge J, Gorski J: A genetic model for the inheritance of pituitary tumor susceptibility in F344 rats. Endocrinology 1981, 109:1708-1714
29. Lloyd HM, Meares JD, Jacobi J: Effects of oestrogen and bromocryptine on *in vivo* secretion and mitosis in prolactin cells. Nature 1975, 255:497-498
30. Hsu S-M, Raine L, Fanger H: Use of avidin-biotin peroxidase complex (ABC) in immunoperoxidase techniques. J Histochem Cytochem 1981, 29:577-580
31. Lloyd RV, Fruhman J: Comparison of peroxidase-anti-peroxidase and avidin-biotin complex methods with radioimmunoassay antibodies. Am J Clin Pathol 1982, 78:795-796
32. Childs GM, Unabia G: Application of the avidin-biotin peroxidase complex (ABC) method to the light microscopic localization of pituitary hormones. J Histochem Cytochem 1982, 30:713-716
33. Asa SL, Ryan N, Kovacs K, Singer W, Marangos PJ: Immunohistochemical localization of neuron-specific enolase in the human pituitary and pituitary adenomas (Abstr). Lab Invest 1983, 48:4A
34. Schmechel D, Marangos PJ, Brightman M: Neuron specific enolase is a molecular marker for peripheral and central neuroendocrine cells. Nature 1978, 276: 834-836
35. Kovacs K, Horvath E, Ezrin C: Pituitary adenomas. Pathol Annu 1977, 12:341-382
36. Duello TM, Halmi NS: Immunocytochemistry of prolactin-producing human pituitary adenomas. Am J Anat 1980, 158:463-469

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