Eur J Cancer Clin Oncol, Vol. 20, No. 6, pp. 829–839, 1984.
Printed in Great Britain.

0277-5379/84$3.00+0.00

Estrone-induced, Prolactin-secreting and Dopamine-sensitive Rat Pituitary Tumor*

MARTIN LIS,† MARC CANTIN,† ANNA-MARIA MARCHISIO,‡ DALAL ELJARMAK† and ROBERT COLLU‡§

†*Clinical Research Institute of Montreal and* ‡*Neuroendocrine Research Laboratory, Pediatric Research Center, Ste-Justine Hospital, Montreal, Quebec, Canada*

Abstract—*Prolactin (PRL)-secreting rat pituitary tumors were induced in female Fisher 344/Lis rats by s.c. implants of estrone (E_1) pellets. Tumor growth was relatively fast and reached about* 100 mg *within* 2 months. *Ovariectomy at the time of E_1 implants seemed to accelerate the growth of the tumors. Tumor cells in primary culture produced mainly PRL, while growth hormone (GH) release was about* 2% *of PRL production and the release of some other pituitary hormones did not exceed* 1% *of PRL values. Tumor cells were found to have high-affinity dopamine (DA) receptors. The addition of DA* in vitro *at* 10^{-10} M *concentration stimulated PRL release, while at* 10^{-6} M *concentration it inhibited the release of the hormone by more than* 50% *of control values. Histological, immunohistochemical and electron microscopical studies demonstrated the tumor to be composed mainly of maximally stimulated mammotrophs.*

INTRODUCTION

PROLACTIN (PRL) secretion is under inhibitory control by dopamine (DA) secreted into hypophyseal portal vessels from dopaminergic neurons [1]. DA receptors have been characterized in the homogenates of the anterior pituitary [2]. In rat pituitary cells DA in low concentrations (1–100 pM) stimulated PRL release, while higher concentrations were inhibitory [3].

The PRL-secreting, transplantable rat pituitary tumors and the cell lines derived from such tumors were reported, however, as non-responsive to DA or dopaminergic drugs or responsive only to very high concentrations of the drugs [4–9]. It is claimed that the lack of DA effect on PRL secretion is due to the absence of high-affinity DA receptors [10].

In the present study we report on an experimental rat pituitary tumor induced by estrone (E_1) which shows complete responsiveness to DA. With this tumor, both the stimulatory effect of low concentrations of DA as well as the inhibitory effect of high concentrations of the neurotransmitter on PRL release could be easily demonstrated *in vitro* using primary cultures of tumor cells. The inhibitory action of dopaminergic agents could also be shown *in vivo* in tumor-bearing animals.

MATERIALS AND METHODS

Animal experiments

Selectively inbred substrains of Fisher 344/Lis rats were maintained for over 20 generations, in the Clinical Research Institute of Montreal, by sibling mating. When female animals reached 100 g body wt they were implanted s.c. with 10 mg of E_1 packed inside a section of silicone tubing (Silastic®, Dow Corning). Unless stated otherwise, all the animals were ovariohysterectomized under ether anesthesia at the time of E_1 implant. The animals were killed by decapitation 2–4 months after the E_1 implant, blood was collected from the trunk using EDTA as anticoagulant and pituitary tumors were harvested.

Primary culture of tumoral cells

Tumor cells were separated and dispersed as previously described [11, 12] using a mixture of dispase, collagenase, deoxyribonuclease and chicken serum. Dispersed cells were attached overnight to polylysine-coated, 24-well plastic

Accepted 9 November 1983.
*Supported in part by the NCI of Canada and by the MRC of Canada.
§To whom correspondence should be addressed at: Neuroendocrine Research Laboratory, Centre de Recherche, Hôpital Ste-Justine, 3175 Chemin Ste-Catherine, Montréal, Québec, Canada H3T 1C5.

cell culture dishes (Costar) in a DME medium containing 5% fetal bovine serum and supplemented as previously described [11] at a concentration of $2–4 \times 10^5$ cells/well. Cell viability, as verified by the erythrosin B dye exclusion test, was higher than 90% after the dispersion procedure. In the incubations where hormone release was measured the cells were washed and incubated in the same medium as above except that the fetal bovine serum was replaced by bovine serum albumin (0.5 mg/ml). Test substances were added as indicated in the results.

In vivo *experiments*

To verify *in vivo* the inhibitory effect of dopaminergic agents on PRL secretion by pituitary tumors, six tumor-bearing animals chronically implanted in the jugular vein with a polyethylene cannula were injected s.c. with apomorphine 5 mg/kg at time 0. One-milliliter blood samples were collected in heparinized tubes before and 5, 15, 30, 45 and 60 min after apomorphine. Red blood cells were immediately separated by centrifugation, resuspended in 1.0 ml of saline and reinjected after the subsequent sampling. Plasmas were stored at −20°C until assayed for PRL.

Light microscopy

For light microscopy the hypophyses were fixed by immersion in Bouin fluid for 24 hr and embedded in paraffin. Five-micrometer sections were stained by the periodic acid Schiff technique (PAS).

Immunocytochemistry

Five-micrometer sections were stained according to the unlabeled antibody technique of Sternberger [13], as already described [14]. NIAMDD rat PRL antiserum was used at dilutions from 10^{-2} to 10^{-4}, with exposure periods varying from 1 to 48 hr. The specificity of the immunocytochemical reactions was evaluated by means of the following controls: exposure to diaminobenzidine was done without incubation with antiserum; normal rabbit serum was substituted for the antiserum; one of the components of the stain was omitted; or the antiserum was preadsorbed with rat PRL [14].

Electron microscopy

The rats were perfused, under ether anesthesia, with Locke–Ringer fluid followed by 2% glutaraldehyde buffered with cacodylate–HCl as previously described [15]. The tumors were removed, minced and kept in the same fixative for 2 hr at 4°C. After a 12-hr wash in cacodylate buffer the specimens were post-fixed in 2% osmium tetroxide buffered with veronal acetate and embedded in Araldite®. Ultrathin sections were examined with a Philips 300 electron microscope after staining with uranyl acetate and lead citrate [15].

Radioimmunoassay

PRL, GH and luteinizing hormone (LH) were measured using materials and procedures supplied by the NIAMDD Rat Pituitary Hormone Distribution Program. Adrenocorticotropic hormone (ACTH) was measured using synthetic human ACTH (CIBA) as a standard and the antibody 3212/13/G kindly supplied by Dr P. Vecsei, Heidelberg, Germany. This antibody is specific to the N-terminal part of ACTH and its cross-reactivity with α-melanocyte-stimulating hormone (α-MSH) is negligible. β-Endorphin was measured using a highly specific commercially available kit (Immunotech, Montreal; cross-reactivity with β-lipotropic hormone is 15%).

RESULTS

The macroscopic aspect of the pituitary tumor *in situ* is shown in Fig. 1.

Tumor growth

The weight of the pituitary tumor after a period of 2 months following the E_1 implant is presented in Table 1. All the tumors used in subsequent experiments were obtained from rats ovariohysterectomized at the time of E_1 implant. This was done essentially to prevent the effect of high levels of E_1 on the uterus such as enlargement,

Table 1. Effects of various operations on body weight changes, pituitary tumor weight and plasma PRL levels in E_1-treated rats

Operation	Body weight changes (g)	Pituitary tumor weight (mg)	PRL (μg/ml)
None	33.9 ± 12.8*	78.2 ± 28.9	4.8 ± 0.78
Ovariectomy	40.4 ± 12.0	126.6 ± 36.4†	4.2 ± 1.37
Hysterectomy	33.4 ± 9.9	69.9 ± 20.2	4.7 ± 0.97
Ovariectomy and hysterectomy	29.1 ± 13.3	120.2 ± 37.2†	2.1 ± 1.96

*Values are means ± S.E.M. Groups of 10 rats were killed 2 months after E_1 implants.
†$P < 0.05$ by Dunnett's t test.

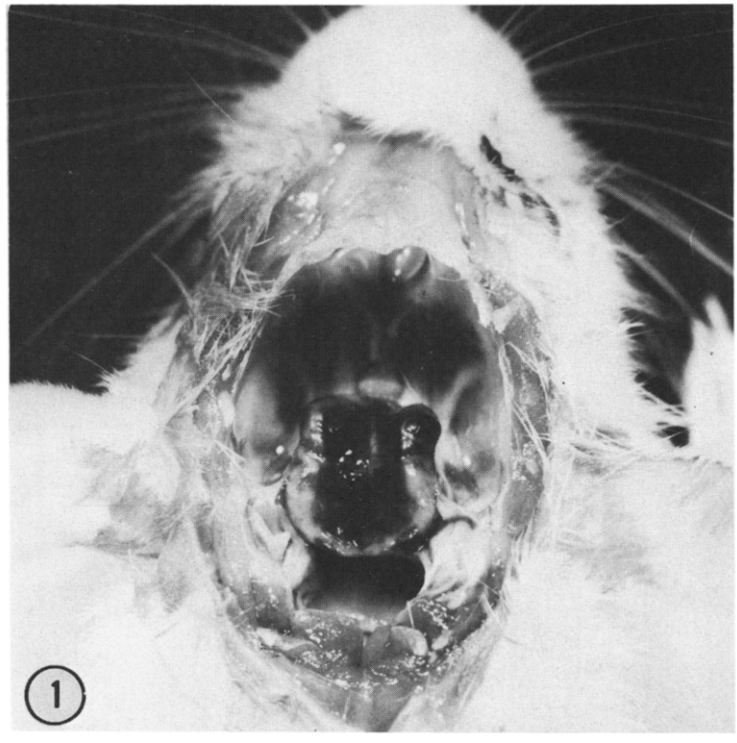


Fig. 1. *Pituitary tumor* in situ 2 months *after* E_1 *implant.*

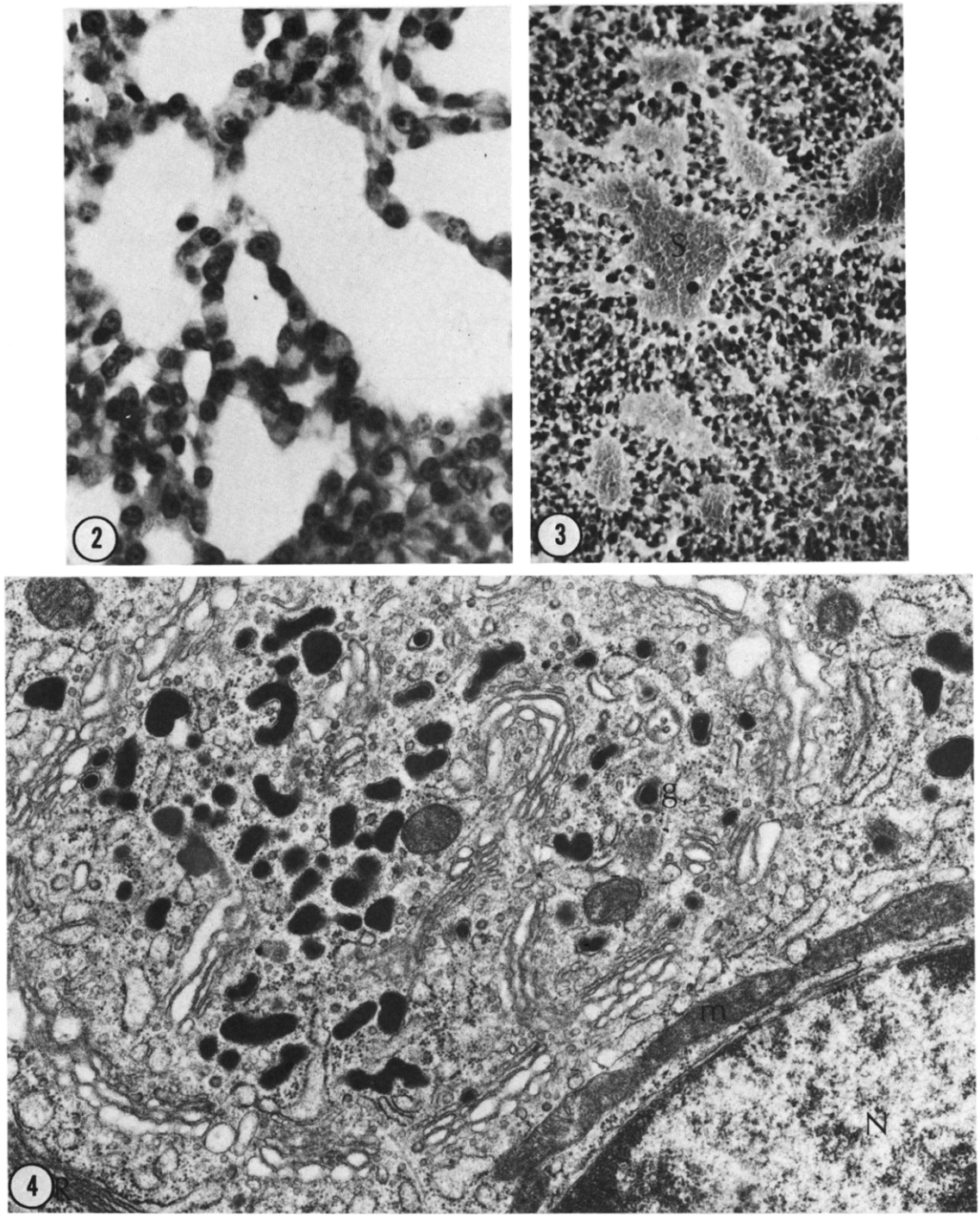


Fig. 2. Histologic appearance of the tumor by light microscopy: cords of tumor cells separated by dilated capillaries filled with red blood cells (PAS–hematoxylin) (×405).

Fig. 3. Section of pituitary tumor stained according to the unlabeled antibody technique of Sternberger (dilution of anti-PRL antiserum 1:1000). *Practically all epithelial cells are reactive. S, dilated sinusoid* (×246).

Fig. 4. Typical, markedly hyperplastic Golgi complex of an estrogen-stimulated mammotroph with numerous saccules and vesicles dispersed in a wide area. G, protogranule; N, nucleus; M, mitochrondrion; R, rough endoplasmic reticulum (×16,016).

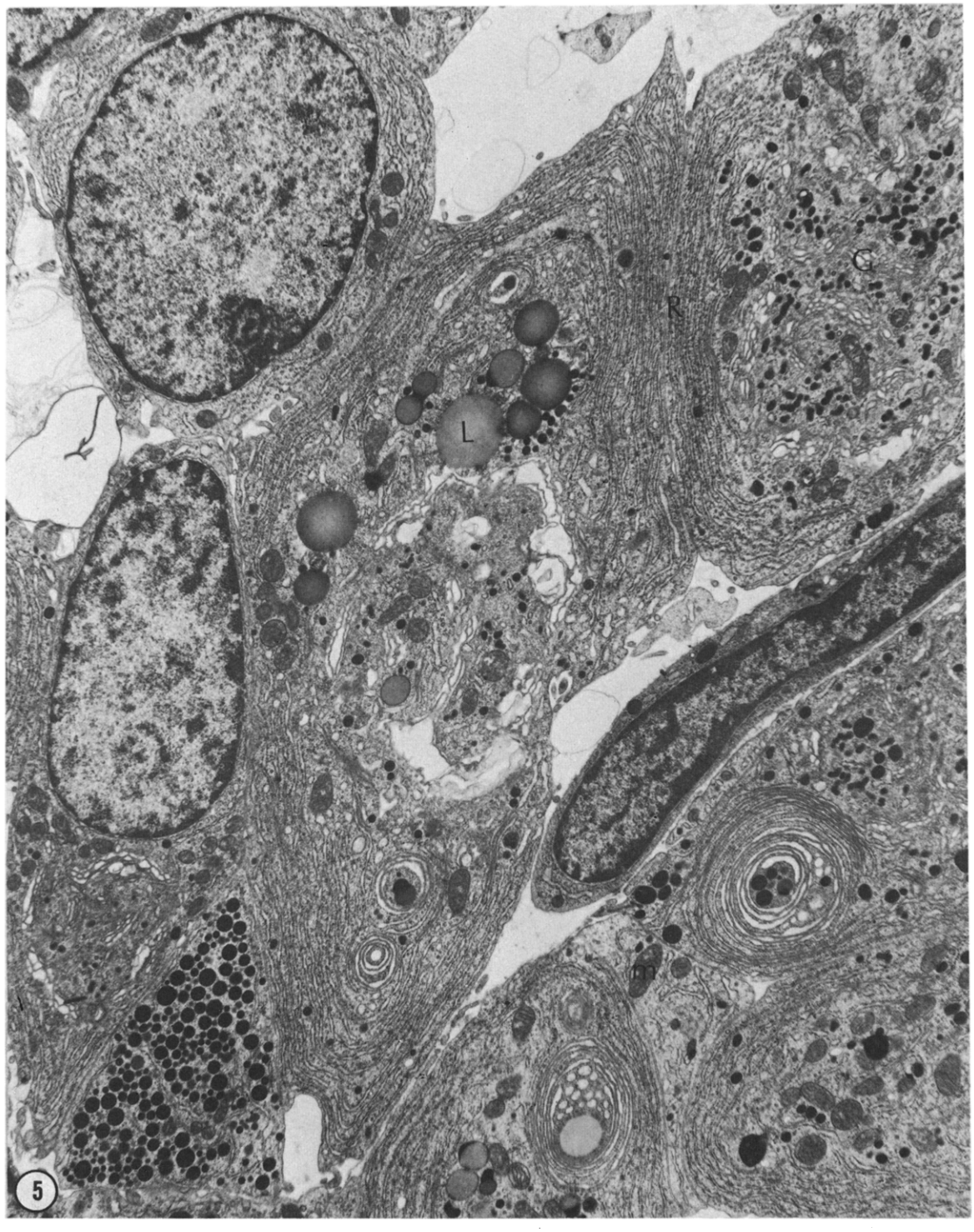
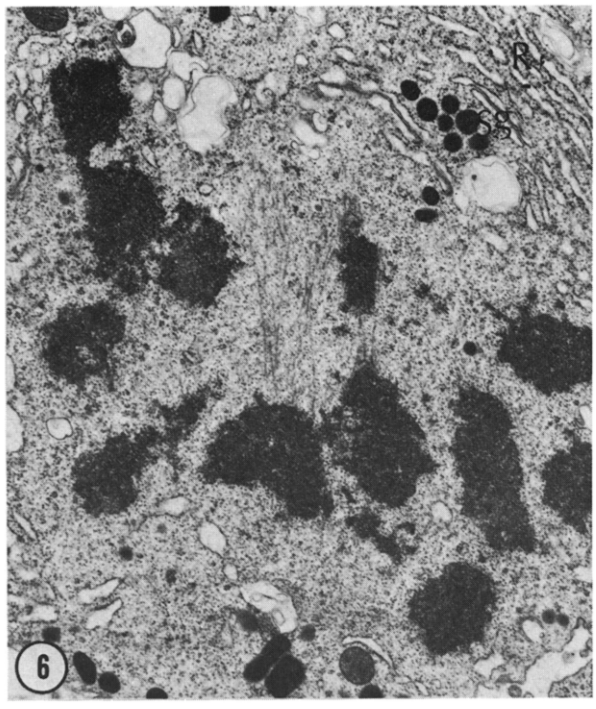
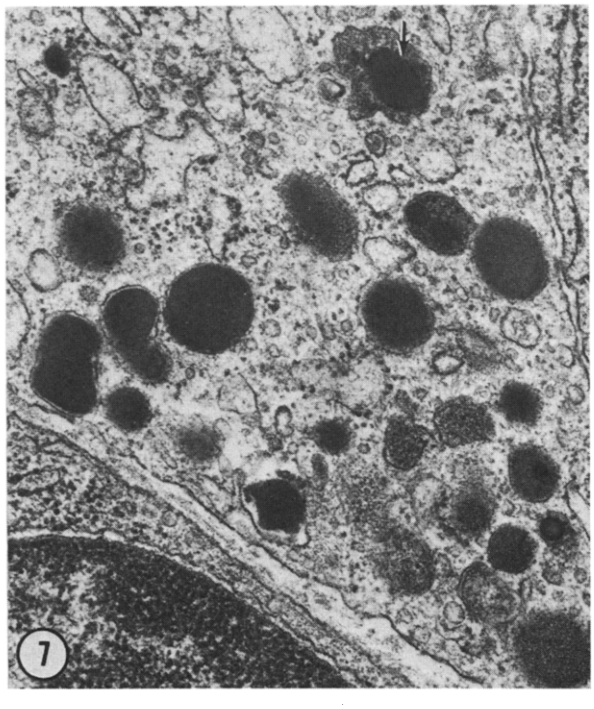


Fig. 5. Several lightly granulated mammotrophs with typical nebenkerne (arrow) and rows of peripheral rough endoplasmic reticulum (R). One cell contains a markedly hyperplastic Golgi complex (G). L, lipid droplet; m, mitochondria. The nuclei are relatively large, with dispersed euchromatin and little heterochromatin (×7395).





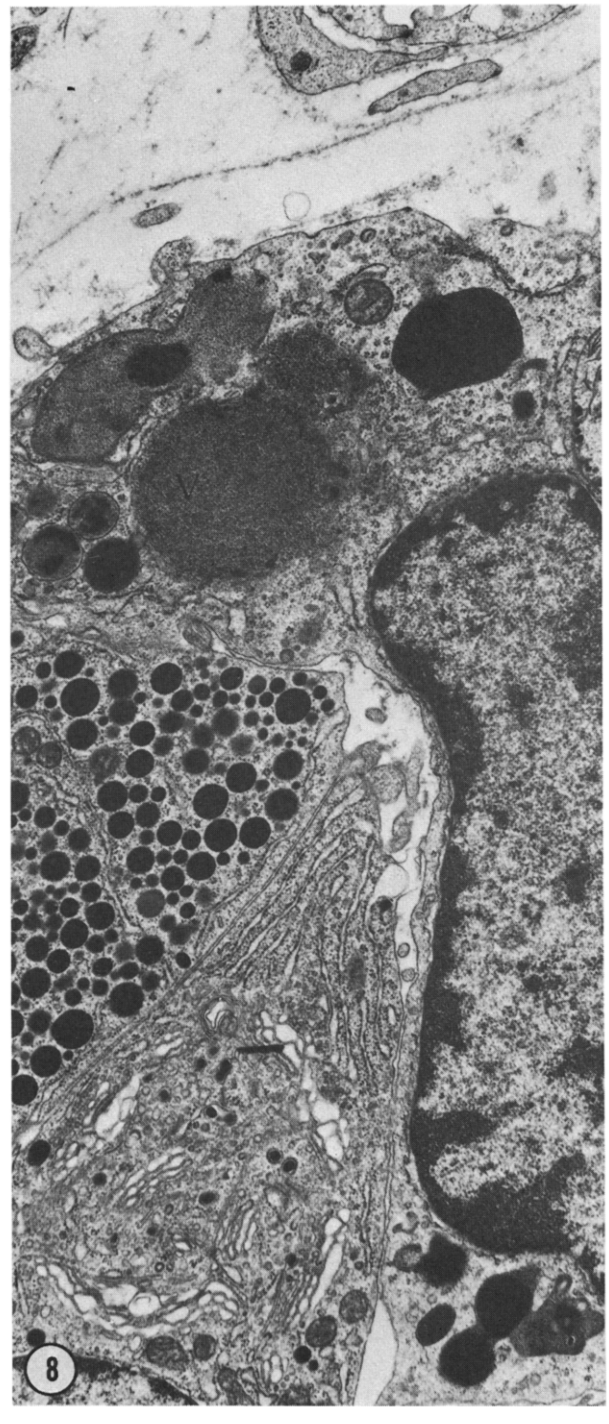


Fig. 6. Mammotroph in mitosis. R, rough endoplasmic reticulum; Sg, secretory granules (×10,208).

Fig. 7. Part of a mammotroph with large, dense secretory granules. A crinophagic vacuole with a typical secretory granule is shown (arrow) (×32,648).

Fig. 8. Mammotroph with nucleus and secretory granules and large autophagic vacuoles (V) containing non-descript debris (×13,939).

hyperemia and usually pyometra. Tumor growth in intact ovariectomized, hysterectomized and ovariohysterectomized rats was compared in Table 1. Surgery had no significant effect on body weight or on plasma PRL concentrations. Ovariectomy, however, stimulated tumor growth.

All the numerous attempts to transplant tumors to syngeneic rats, with or without E_1 implants, have been unsuccessful so far. Tumor cells could be easily maintained in primary culture and kept producing PRL for more than 3 weeks. Numerous attempts to grow the tumor as a permanent cell line using different media supplemented with various growth factors have also been unsuccessful so far.

In vitro *secretion by tumoral cells*

Tumor cells in primary culture produced mostly PRL, with rather small quantities of other peptides, as shown in Table 2. GH was the second most abundant peptide. On a molar basis, however, GH represented approximately 2% of PRL production. Other peptide secretions are less than 1% of that of PRL.

Effects of DA on PRL release in vitro

As shown in Table 3, DA at 10^{-10} M concentration stimulated PRL secretion while at 10^{-6} M concentration was inhibitory and reduced the secretion of the hormone by approximately 50%. A more detailed analysis of the effects of dopaminergic drugs on *in vitro* PRL secretion is the subject of a separate publication [Eljarmak *et al.*, submitted for publication].

Table 2. In vitro *secretion of pituitary hormones by tumor cells*

Peptide	Secretion per 10^6 cells
PRL	1.975 ± 0.13* μg (12)
ACTH	127.080 ± 30.30 pg (8)
GH	42.500 ± 6.50 ng (8)
LH	25.300 ± 4.60 ng (4)
β-Endorphin	5.980 ± 0.79 ng (6)

*Values are means ± S.D. (No. of incubations). The cells were incubated for 4 hr with the exception of ACTH, where the cells were incubated for 1.5 hr.

Table 3. Effects of DA on PRL release by tumor cells in vitro

Treatment	PRL (μg/10^6 cells)
Control	1.69 ± 0.12* (8)
DA 10^{-10} M	2.13 ± 0.20† (8)
DA 10^{-6} M	0.65 ± 0.13† (8)

*Values are means ± S.E.M. (No. of incubations).
†$P < 0.001$ vs control by Student's t test.

In vivo *experiments*

The effects of apomorphine, a DA agonist, on PRL secretion *in vivo* by tumor-bearing rats are shown in Table 4. The s.c. administration of 5 mg/kg of the drug induced a drastic and rapid reduction of circulating PRL that reached 25% of baseline values at 60 min.

Light microscopy

The tumor could be characterized (Fig. 2) as a congested, focally hemorrhagic, spongy structure consisting of epithelial cords separated by large empty spaces lined with endothelium. The epithelial cells contained only a few PAS-positive granules. Brown granules not stained by the PAS were also present. Some macrophages containing abundant PAS-positive granules were also present amongst the epithelial cells, mostly near dilated capillaries.

Immunocytochemistry

Cords of positive cells (Fig. 3) were separated by large, unreactive, empty spaces, corresponding to enlarged capillaries. The best results were obtained after a 48-hr exposure to PRL antiserum at 1:1000 dilution. Practically all cells contained varying amounts of brown precipitate, frequently in a microgranular distribution. The nuclei were unstained. All controls showed a negative reaction. Pre-adsorption of the antiserum with 5 ng of PRL consideraly diminished the reaction and preadsorption with 5 μg of PRL completely abolished it.

Table 4. Effects of apomorphine on PRL secretion in vivo

Time (min)	PRL (μg/ml)
0	6.2 ± 0.5*
5	5.9 ± 0.2
15	5.0 ± 0.4
30	3.4 ± 0.8†
45	2.6 ± 0.9†
60	1.6 ± 0.6†

*Values are means ± S.E.M. Six rats bearing a pituitary tumor and chronically cannulated in the jugular vein were utilized. Apomorphine 5 mg/kg was injected s.c. at time 0.
†$P < 0.001$ vs time 0 min by paired Student's t test.

Electron microscopy

The large majority of epithelial cells were mammotrophs identified by their dense, large and polymorphous granules. Desmosomes were often seen at all stages. Many cells were highly granulated and possessed a large paranuclear Golgi complex made up of several rows of saccules and vesicles, often containing protogranules (Fig. 4). Immature secretory granules, seemingly formed by fusion of several smaller units, had an irregular shape, often with a clear space between the dense core and the peripheral membrane. The rough endoplasmic reticulum was abundant, often subplasmalemmal and formed in many cells typical whorls called 'nebenkerne' (Fig. 5). Mitochondria were scarce and often of irregular shape. Lipid droplets were sometimes present in the cytosol. The nuclei were large, contained little visible eu- and heterochromatin and had prominent nucleoli. Mitoses were frequent in cells containing typical granules (Fig. 6). Crinophagy (presence of secretory granules in various phases of digestion inside phagolysosomes) was observed in several mammotrophs (Fig. 7). Large phagolysosomes containing amorphous debris were also present in some of these cells (Fig. 8).

DISCUSSION

Estrogen-induced pituitary tumors have been described as early as 1936 [16]. Estrogens increase both PRL synthesis [17] and release [18]. Their effect is probably direct since it can be demonstrated *in vitro* using primary cultures of anterior pituitary cells [17–19]. Pre-incubation with estrogens reversed the inhibitory effect of DA agonists *in vitro* [19], suggesting that estrogens may decrease the pituitary sensitivity to dopaminergic factors. However, this does not exclude the possibility that estrogens could also influence PRL secretion through some mechanisms mediated by the hypothalamus [20, 21]. Tumor cells used in our study retained their responsiveness to DA comparable to normal pituitary cells, as previously described [3]. The cells are obtained from rats chronically treated with E_1 implants. It may be that the short period of time (3–5 days) in culture completely restored the responsiveness to DA of the tumoral cells used in this study. However, the administration *in vivo* of apomorphine, a dopaminergic agonist, drastically inhibited PRL release in E_1-treated, tumor-bearing animals. In addition, recent pharmacological studies performed in our laboratory have indicated the presence in tumoral mammotrophs of dopaminergic receptors having a density and affinity comparable to those found in normal mammotrophs [Eljarmak *et al.*, submitted for publication]. Several estrogen-induced pituitary tumors have been developed, usually in the form of a transplantable tumor, in inbred rat strains [22]. Many transplantable pituitary tumor lines have been maintained for more than 25 yr and retain a secretory capacity. While various pituitary hormones such as ACTH and GH [22] are sometimes secreted by these tumors, PRL seems to be the most persistent and the most abundant secretory product. However, transplantable tumors, as well as the cell lines developed from them [4, 6, 8, 10], have lost, either completely or at least partially, their ability to respond to either DA or DA-like drugs.

The loss of high-affinity DA receptors seems to be correlated with the ability of these tumors to grow as autonomous transplants or as permanent cell lines *in vitro*. The meaning of this relationship remains to be elucidated. Primary tumors induced by estrogens grew faster *in situ* than tumors developed from grafted pituitary tissue [23]. However, tumors from grafted pituitaries were much more successful as subsequent transplanted tumors than pituitary tumors grown *in situ* [24].

E_1 has been extensively used to develop many lines of transplantable tumors of different tissues in Nb rats [24, 25]. From the limited number of data published on E_1- or stilbestrol-induced pituitary tumor growth, and taking into account the differences in the methods and dosage [24, 26], it can be concluded that the substrain of Fisher 344/Lis rats appears to be very sensitive to E_1 treatment. As shown in Table 1, a pituitary tumor growth rate of about 100 mg in 2 months could easily be obtained with an incidence of successfully induced tumors of almost 100%. A much slower tumor growth has been reported in similar experiments using other rat strains, including Fisher 344 [22, 24, 26], although more recently, Wiklund *et al.* [27] have reported the rapid induction of pituitary tumors with diethylstilbestrol in this strain of animals.

Histological, histochemical and electron-microscopical studies revealed that tumor cells of the present report had all the characteristics of maximally stimulated mammotrophs. They secreted large quantities of PRL, as previously described with estrogen-treated rats [28–30]. Large amounts of rough endoplasmic reticulum, often in the form of nebenkerne, an extremely developed Golgi complex often containing progranules, and polymorphous, dense, large granules were present. A subpopulation of secretory granules, often located in the vicinity of the Golgi complex and made up of aggregated and condensed reticulum, are a well known characteristic of estrogen-induced mammotroph

adenomas and are believed to be a sign of high secretory activity [31].

Immunohistochemical staining revealed the presence of immunoreactive PRL in the cytoplasm of most cells. The intensity of immunostaining was uneven and varied from cell to cell. The absence of immunoreactivity in controls, particularly after absorption of the antiserum with its antigen, excluded the possibility of a non-specific reaction [13].

Crinophagy, demonstrated previously in PRL-secreting cells [32], was evident in many tumoral cells of the present study. The large autophagic vacuoles observed in some cells might correspond, at the light-microscopic level, to pigment granules, which may be PAS-positive or -negative, depending on the amount of glycoprotein present [15]. Abundant pigment granules, mostly localized in macrophages but also in some mammotrophs, have been observed in the spontaneous PRL adenomas of aged rats [33].

The replacement of the vast majority of the cells of the anterior lobe of the hypophysis by mammotrophs as a consequence of estrogen stimulation is undoubtedly due to the selective mitotic proliferation of these cells [34]. The transformation of somatotrophs into mammotrophs has also been suggested to occur in similar experimental models [35].

In conclusion, the type of primary rat tumor presented in this study is a model for prolactinomas with high-affinity dopaminergic receptors. Most of the human prolactinomas, as with the present tumor, are sensitive to the inhibitory effect of dopaminergic drugs [36]. Some human prolactinomas are also reported to have decreased sensitivity to dopamine. The degree of dopamine responsiveness in these cases has shown a good agreement between *in vivo* results and *in vitro* data obtained with primary cultures of cells from surgically removed prolactinomas [26]. It would be of great interest to note the existence of a relationship between the prolactinoma responsiveness to dopamine and the clinical course of the tumor after treatment. In different rat models the loss of high-affinity dopaminergic receptors is, as a rule, associated with the ability of the tumor cells to grow in culture as a permanent cell line [8] or to grow as a transplantable tumor [6].

The tumor model introduced in this report is the first described with dopaminergic responsiveness similar to that of the normal pituitary gland. The advantages of this model are the relative ease and rapidity of induction, and the suitability for *in vitro* experiments. These are facilitated by a relative ease of cell isolation and dispersal, and also by the large size of the tumor, which contains a rather uniform cell population.

Acknowledgements—The authors wish to thank Dr P. Vecsei, Heidelberg, Germany, for the gift of ACTH antibody and Dr A. F. Parlow and the Pituitary Agency of the NIAMDD for the gift of PRL, GH and LH radioimmunoassay reagents. The excellent technical assistance of A. Chen, M. Ballak and H. Provost, and the secretarial help of S. Tassé and L. Lalonde are greatly appreciated.

REFERENCES

1. MacLeod RM. Regulation of prolactin secretion. In: Martin L, Ganong WF, eds. *Frontiers in Neuroendocrinology*. New York, Raven Press, 1976, Vol. 4, 169–194.
2. Caron MG, Beaulieu M, Raymond V *et al.* Dopaminergic receptors in the anterior pituitary gland. *J Biol Chem* 1978, **253**, 2244–2253.
3. Denef C, Manet D, Dewals R. Dopaminergic stimulation of prolactin release. *Nature* 1980, **285**, 243–246.
4. Bang S, Gautvik KM. Inhibition of prolactin secretion and synthesis by dopamine, noradrenaline and pilocarpine in cultured rat pituitary tumor cells. *Acta Pharmacol Toxicol* 1977, **41**, 317–327.
5. Dannies TS, Tashjian AH. Growth hormone and prolactin from rat pituitary tumor cells. In: Kruse PF, Paterson MK, eds. *Tissue Culture*. New York, Academic Press, 1973, 561–585.
6. Lamberts SWJ, MacLeod RM. The inability of bromocriptine to inhibit prolactin secretion by transplantable rat pituitary tumors. Observations on the mechanism and dynamics of the auto-feedback regulation of prolactin secretion. *Endocrinology* 1979, **104**, 65–70.
7. MacLeod RM, Lamberts SWJ, Nagy I, Login IS, Valdenegro CA. Suppression of prolactin secretion by the physiological and pharmacological manipulation of pituitary dopamine receptors. In: Gaglia G, Giovanelli NA, MacLeod RM, eds. *Pituitary Microadenomas*. New York, Academic Press, 1980, 37–54.
8. Malarkey WB, Groshong JC, Milo GE. Defective dopaminergic regulation of prolactin secretion in a rat pituitary tumor cell line. *Nature* 1977, **266**, 640–641.

9. Quadri SK, Lu KH, Meites J. Ergot-induced inhibition of pituitary tumor growth in rats. *Science* 1972, **176**, 417–418.
10. Cronin MJ, Faure N, Martial JA, Weiner RI. Absence of high affinity dopamine receptors in GH_3 cells: a prolactin-secreting clone resistant to the inhibitory action of dopamine. *Endocrinology* 1980, **106**, 718–723.
11. Lis M, Hamet P, Gutkowska J *et al.* Effect of N-terminal portion of pro-opiomelanocortin on aldosterone release by human adrenal adenoma *in vitro*. *J Clin Endocrinol Metab* 1981, **52**, 1053–1056.
12. Schiffrin EL, Lis M, Gutkowska J, Genest J. Role of Ca^{2+} in response of adrenal glomerulosa cells to angiotensin II, ACTH, K^+, and ouabain. *Am J Physiol* 1981, **241**, E42–E46.
13. Sternberger LA. *Immunocytochemistry*. New York, Wiley, 1979, 122–130.
14. Lu CL, Cantin M, Seidah NG, Chrétien M. Immunohistochemical localization of human pituitary glycopeptide (HPGP)-like immunoreactivity in the hypothalamus and pituitary of normal and homozygous diabetes insipidus (Brattleboro) rats. *J Histochem Cytochem* 1982, **30**, 999–1003.
15. Cantin M, Benchimol S. Localization and characterization of carbohydrates in adrenal medullary cells. *J Cell Biol* 1975, **65**, 463–479.
16. Gardner WV, Smith GM, Strong LC. An observation of primary tumors of the pituitary. *Am J Cancer* 1936, **26**, 541–547.
17. Lieberman ME, Maurer RA, Gorski J. Estrogen control of prolactin synthesis *in vitro*. *Proc Natl Acad Sci USA* 1978, **75**, 5946–5949.
18. Nicoll CS, Meites J. Estrogen stimulation of prolactin production by rat adenohypophysis *in vitro*. *Endocrinology* 1962, **70**, 272–277.
19. Raymond V, Beauleiu M, Labrie F, Boissier J. Potent antidopaminergic activity of estradiol at the pituitary level on prolactin release. *Science* 1978, **200**, 1173–1175.
20. Kanematsu S, Sawyer C. Effects of intrahypothalamic and intrahypophyseal estrogen implants on pituitary prolactin and lactation in the rabbit. *Endocrinology* 1963, **72**, 243–252.
21. Ratner A, Meites J. Depletion of prolactin-inhibiting activity of rat hypothalamus by estradiol of suckling stimulus. *Endocrinology* 1964, **75**, 377–382.
22. Furth J. Hormones and abnormal growth. Experimental pituitary tumors. *Recent Prog Horm Res* 1955, **11**, 221–255.
23. Welsch CW, Jenkins T, Amenomori Y, Meites J. Tumorous development of *in situ* and grafted anterior pituitaries in female rats treated with diethylstilbestrol. *Experientia* 1971, **27**, 1350–1352.
24. Noble RL, Hochachka BC, King D. Spontaneous and estrogen produced tumors in Nb rats and their behavior after transplantation. *Cancer Res* 1975, **35**, 766–780.
25. Noble RL, Hoover L. A classification of transplantable tumor in Nb rats controlled by estrogen from dormancy to autonomy. *Cancer Res* 1975, **35**, 2935–2941.
26. Tallo D, Malarkey WB. Adrenergic and dopaminergic modulation of growth hormone and prolactin secretion in normal and tumor bearing human pituitaries in monolayer culture. *J Clin Endocrinol Metab* 1981, **53**, 1278–1284.
27. Wiklund J, Wertz N, Gorski J. A comparison of estrogen effects on uterine and pituitary growth and prolactin synthesis in F344 and Holtzman Rats. *Endocrinology* 1981, **109**, 1700–1707.
28. Farquhar MG, Furth J. Electron microscopy of experimental pituitary tumors. *Am J Pathol* 1959, **35**, 688–715.
29. Hymer WC, McShan WH, Christiansen RG. Electron microscopic studies of anterior pituitary glands from lactating and estrogen treated rats. *Endocrinology* 1961, **69**, 81–90.
30. Schelin V, Lundin PM, Bartholson L. Light and electron microscopic studies on an autonomous stilboestrol-induced pituitary tumor in rats. *Endocrinology* 1964, **75**, 893–900.
31. Lundin PM, Schelin V. Light and electron microscopical studies on the pituitary in stilboestrol-treated rats. *Acta Path Microbiol Scand* 1962, **54**, 66–74.
32. Farquhar MG. Secretion and crinophagy in prolactin cells. In: Dellman HD, Johnson JA, Klachko DM, eds. *Comparative Endocrinology of Prolactin*. New York, Plenum Press, 37–94.
33. Kovacs K, Horvath E, Ilse RG, Ezrin C, Isle D. Spontaneous pituitary adenomas in aging rats. A light microscopic, immunocytological and fine structural study. *Beitr Pathol* 1977, **161**, 1–16.

34. Gersten BE, Baker BL. Local action of intrahypophyseal implants of estrogen as revealed by staining with peroxidase-labeled antibody. *Am J Anat* 1970, **218**, 1–20.
35. Stratmann IE, Ezrin C, Sellers EA. Estrogen-induced transformation of somatotrophs into mammotrophs in the rat. *Cell Tissue Res* 1974, **152**, 229–238.
36. Lachelin GCL, Abu-Fadil S, Yen SSC. Functional delineation of hyperprolactinemic amenorrhea *J Clin Endocrinol Metab* 1977, **44**, 1163–1174.