Spontaneous Pituitary Tumors in the Wistar/Furth/Ico Rat Strain

An Animal Model of Human Prolactin Adenoma

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Twenty-three spontaneous pituitary tumors in 58 rats of Wistar/Furth/Ico strain were studied. The incidence is 38% in rats older than 10 months; it rises with age, with a maximum at 28–32 months (68.7%) and is higher in females (71.4%) than in males (35%) over 17 months. Light-microscopic and immunocytochemical studies revealed 20 prolactinomas in 19 rats (19/58, 32.7%) and 3 spongiocytic nonimmunostaining adenomas (3/58, 5.2%). The prolactinoma is often hemorrhagic. The cells, often arranged in sheets and agranular, are mostly positive with anti-rat prolactin (rPRL) serum. They have few polymorph granules and a well-developed rough endoplasmic reticulum. In the spongiocytic adenoma, the cells are arranged in cords. Their cytoplasm is slightly vacuolated. In prolactinoma-bearing rats, the mean plasma PRL value was 213 ± 72.5 μg/l (SEM) (N = 15 ± 1.8 μg/l [SEM]). A linear correlation was found between the logarithm of the tumoral pituitary weight or of the tumor size and the logarithm of the prolactinemia. Because of the analogies between these rat prolactinomas and 57 human prolactinomas, the Wistar/Furth/Ico rat strain is considered as a good animal model. (Am J Pathol 1982, 109:57–70)

SPONTANEOUS PITUITARY TUMORS in rats of various strains have been repeatedly reported in the literature;[1–26] however, this species has not been used to full advantage in pituitary adenoma research because of an inadequate knowledge of the spectrum of the tumors and their character in the available inbred rat strains. In about 1949–1951, Furth originated the Wistar/Furth strain (W/Fu) from a commercial pen-bred stock from the Wistar Institute.[27,28] Our knowledge of this strain is very poor. Kim et al[29] reported an incidence of 27.3% of pituitary tumors in aging females, and Ito et al[30] 60% of these tumors in aging males. Recently, Prysor-Jones and Jenkins[31] found 69.2% and 6.1% in females and males, respectively. Most of these pituitary tumors are prolactinomas with hyperprolactinemia. No global study was done utilizing the new techniques of pituitary investigation and prolactin radioimmunoassay (RIA).

We undertook our study to determine the incidence as well as the histologic, immunocytochemical, ultrastructural, and hormonal characteristics of spontaneous pituitary adenomas of the W/Fu strain, with special reference to microadenomas. We compared the rat pituitary prolactinomas with 57 human prolactinomas to determine whether the Wistar-Furth rat strain was a good animal model of the human prolactinoma.

Materials and Methods

Wistar/Furth/Iffa Credo (W/Fu/Ico) Rats

Materials

The original couple of this W/Fu strain came from Microbiological Associates, Inc., Laboratory, Bethesda, Maryland. From 1971, this strain has been in-

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bred by more than 42 consecutive brother-sister matings at Iffa Credo (69210, Saint-Germain-sur-l'Arbresle, France). The females bore an average of 5.3 litters. The mean age of the first litter was 92 days. Sterility was rare (6%), and mortality was low (11%). Mean longevity was 30–32 months.

Fifty-eight rats maintained in a barrier breeding unit (Iffa Credo) were delivered to our laboratory at 7 months of age. The males and females were maintained separately 2 to a cage, at a temperature of 22 ± 3 C. These rats were fed a standard rat diet (Souriffarat, Iffa Credo) and had tap water *ad libitum*. Three animals, 2 males and 1 female, died at 17, 28, and 29 months; and autopsies were performed soon thereafter. Fifty-five rats, 27 males and 28 females, were decapitated, and autopsies were performed at determined ages. These 58 animals were divided into 4 age groups: Group I, 10–12 months (9 males and 8 females); Group II, 17–18 months (7 males and 6 females); Group III; 24–25 months (6 males and 6 females); Group IV, 28–32 months (7 males and 9 females). None of the animals had undergone any treatment.

Histologic Examination

After decapitation, the pituitary glands were immediately removed and weighed. For light-microscopic examination, each pituitary was fixed in Gérard's fluid (Bouin-Hollande sublimate without acetic acid), cut entirely and serially at 4 μ of thickness, and mounted on glass slides. In order to show any microadenomas, 3 slides were stained with Masson's trichrome, Herlant's tetrachrome, and PAS–orange G at the beginning, in the middle, and at the end of the series of slides. The size of the tumor was expressed in square millimeters (maximum length × maximum diameter). The comparison of the pituitary weights and tumor size was expressed with the standard error of mean (SEM), and eventually the Student t test[32] was used for testing the significance of differences between males and females. For immunocytochemical localization of prolactin (PRL), growth hormone (GH), and corticotropin (ACTH), the immunofluorescence technique was used according to the indirect method of Weller and Coons; details of the method are reported elsewhere.[33] The following rabbit antiserums were used: anti-human GH, anti-rat PRL, anti-$^{17-39}$ACTH (M. P. Dubois, who prepared these antiserums, has assessed the immunologic specificity as reported previously[34]). In addition, control sections were treated 1) with antiserums inactivated by the addition of excess antigens; 2) with normal rabbit or normal human serums as substitutes for the specific antibodies; 3) incubated with conjugated sheep anti-rabbit IgG serum only. Four large tumors were studied by electron microscopy. Tissue fragments were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer and postfixed in 2% osmium tetroxide. They were embedded in araldite. The ultrathin sections, cut with a Reichert OMU 3 ultramicrotome, were contrasted with uranyl acetate and lead citrate. They were examined with a JEOL type JEM 7 electron microscope.

The adrenals, the gonads, and any tumor or pathologic tissue were also removed, weighed, and fixed in Gérard's fluid. The sections were stained with Masson's trichrome.

RIA

From 55 rats, the concentration of plasma prolactin was measured by double-antibody RIA with the use of the material supplied by the National Institute of Arthritis, Metabolic and Digestive Diseases (NIAMDD). Results are expressed in terms of the NIAMDD standard prolactin RP_1.

Human Pituitary Prolactinomas

Since 1971, we have studied 252 pituitary adenomas, of which 57 were prolactinomas, including 11 microadenomas (diameter <1 cm). These 57 tumors were removed by the transsphenoidal route from 36 women and 21 men by Prof. Goutelle, Prof. Fischer, and Dr. Perrin (Hôpital Neurologique, Lyon).

Each tumor was studied with the use of light microscopy, immunocytochemistry (anti-hGH, anti-hPRL, anti-$^{17-39}$ACTH, anti-β-hFSH, anti-hLH, anti-β-endorphin were tested), and electron microscopy with the same methods as the rat pituitaries. For 46 patients, the mean value of plasma prolactin was established before surgery by RIA (normal value <30 μg/l).

Results

Wistar/Furth/Iffa Credo (W/Fu/Ico) Rats

Histologic Data

Pituitary Findings

Gross Inspection: Forty-five pituitaries seemed normal. However, in 8 of them, in serial sections we detected a microadenoma. In 3 cases, a small adenoma was suggested by the irregular surface of the gland and/or a dark red spot. Eleven large tumors were roughly spherical and circumscribed. They protruded from the sella and compressed the brain. One surrounded the cerebellum on either side but did not invade it. They were soft and friable, often extremely

congested and dark red. In 6 cases (Cases 10, 14, 18, 19, 20, and 38) the entire pituitary gland seemed to be replaced by the tumor. In the other cases, the tumor was clearly distinguished from the nontumoral pituitary. In rats with neither gross nor microscopic tumors, the pituitary weights averaged 14.9 ± 0.7 mg (SEM) (range, 10–21). The mean values of the pituitary weight were significantly different ($P < 0.01$) in the males (13.1 ± 0.8 mg) and in the females (17.3 ± 0.8 mg). The pituitaries with microadenomas (size, <1 sq mm) and with macroadenomas averaged 17.5 ± 1.5 mg (range, 11–28) and 97.6 ± 29 mg (range, 17–289), respectively.

Microscopic Findings: In the *normal pituitaries*, the GH cells (orangeophilic with Herlant's tetrachrome), which were positive with anti-hGH serum, were more numerous in the male than in the female glands. In the females, the prolactin cells (often erythrosinophilic with Herlant's tetrachrome), which reacted with the anti-rPRL serum, were very numerous (50–70%). Both males and females presented the same number of scattered cells reacting with the anti-$^{17-39}$ACTH serum. Twenty-three *pituitary adenomas*, 12 microadenomas (Figure 1), and 11 macroadenomas (Figure 2), were recognized in 22 animals. One rat had 2 microadenomas. The tumor size varied from 0.17 sq mm to 28 sq mm, with an average of 3.6 ± 1.4 sq mm. A linear correlation ($r = 0.92$) was found between the logarithm of the pituitary weight and the logarithm of the tumor size. The localization of these microadenomas was variable: some were in the center of one lobe, some in the ventral part, and some near the intermediate lobe. They were distinguishable from the surrounding hypophysial tissue by their arrangement in mainly solid sheets, and by blood-filled spaces which were never lined by endothelial cells. In 4 cases, circumscribed hyperplasia of hyperactive cells with large nuclei and nucleoli was not considered a microadenoma, because it was observed in only a few serial sections and presented the normal cordonal pattern with several types of cells (Figure 3). Pituitary weights were at the upper limit of normal in 3 of them (19, 20, and 21 mg). In the 6 largest tumors, only some cords of normal pituitary cells remained. In one case (Case 38), the surrounding pituitary tissue showed greatly dilated capillaries. In the others it was normal: hyperactivity and hyperplasia of cells was not seen. Apart from some details (size and hemorrhagic character), the 12 microadenomas were not different from the 11 macroadenomas. Immunofluorescence revealed 20 prolactinomas, of which 10 were microadenomas, and 3 nonimmunostaining adenomas, of which 2 were microadenomas.

In the *prolactinomas*, the cells were arranged in mainly solid sheets or, rarely, in cords. The capillaries lined by endothelial cells were very few, in contrast with the many round blood-filled spaces, which were never lined by endothelial cells. In 7 cases, these blood spaces were extensive and associated with large hemorrhagic areas such that we called them "hemorrhagic prolactinomas" (Figure 2). In these cases, the accumulation of hemosiderin pigment was readily noticeable near the hemorrhagic areas, in adenoma cells or in macrophages. In Case 24, small colloid-filled cavities were formed. There was a variation in cellular size and tinctorial characteristics from adenoma to adenoma. In one adenoma (Case 14), there

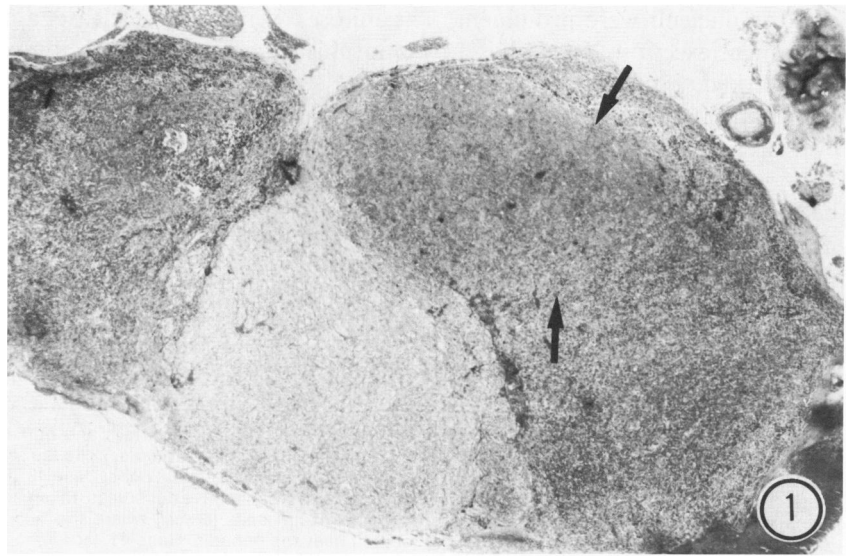


Figure 1 — Microprolactinoma in the right lobe, seen by gross inspection (irregular surface). Case 22. (Masson's trichrome, ×40)

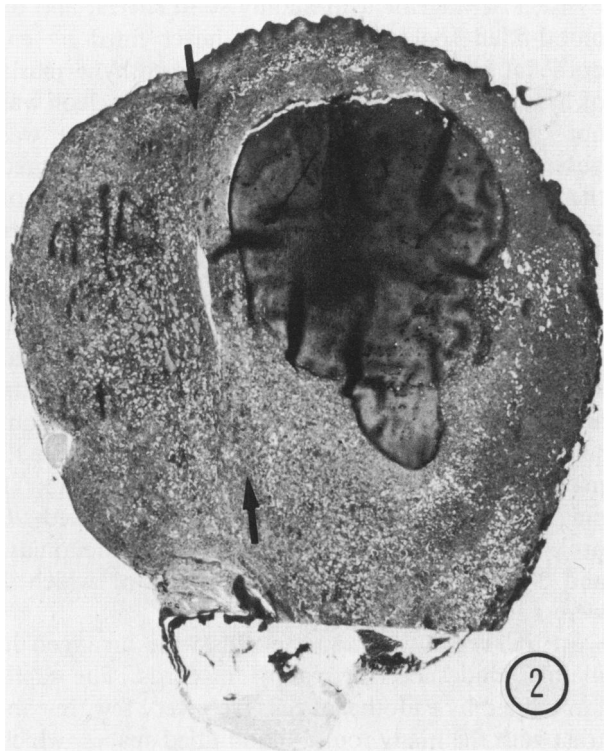
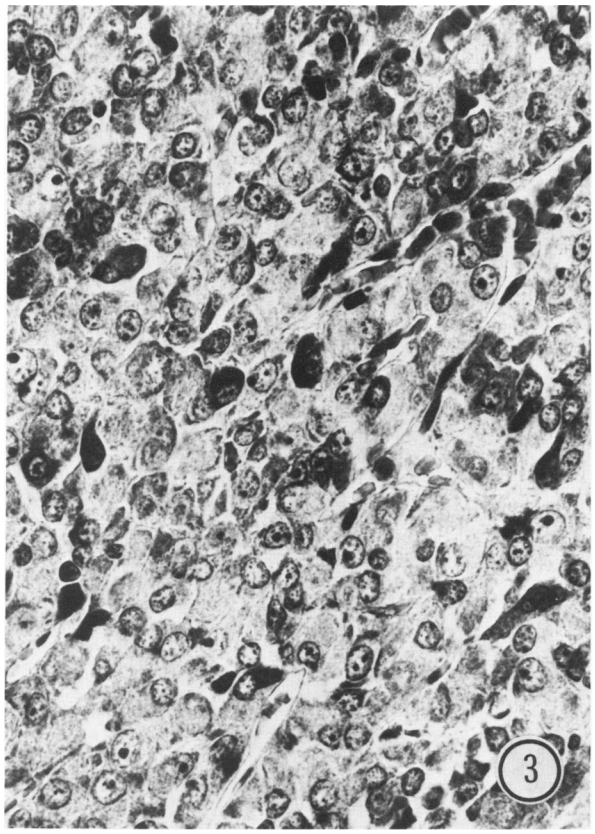


Figure 2—Hemorrhagic macroprolactinoma. Apart from a few cell cords (*arrows*), the normal pituitary is replaced by the tumor. Case 18. (PAS-orange G, ×15)

was an area of large, clear prolactin cells and another of small basophilic ones. In 14 cases, the cells were small, with a basophilic rim of cytoplasm (Figure 4). In 6 cases, the cytoplasm of the cells were abundant, agranular, and PAS-negative (Figure 5). Only 2 adenomas showed erythrosinophilic granulations with Herlant's tetrachrome. The negative image of the Golgi complex was often seen. The nuclei were large, and the nucleoli were prominent. The mitoses were infrequent except in 4 cases, and pleomorphism was not marked, except in 2 cases. No definite signs of malignancy and no connective tissue capsule were ever observed in any of the cases studied (Figure 5). However, Case 19 was called "invasive prolactinoma" because it surrounded the adjacent blood vessels, nerves, and bone; but no infiltration of neighboring tissues and no metastases were seen. It was also "uncommon," because, although it was the largest tumor studied, no hemorrhage was observed. The prolactin cells were very small, with many atypical mitotic figures and hyperchromatic nuclei with small nucleoli. Many giant cells were also observed. In 3 rats (Cases 19, 21, and 28) with lymphoid or mononuclear-cell leukemia, leukemic cells infiltrated the tumor. By immunofluorescence, the percentage of positive cells with the anti-rPRL antibody varied from tumor to tumor between 30% and 100%. In most cases the cells were small and the whole cytoplasm was positive, but the reaction was weaker than in the normal prolactin cells. Sometimes in the large cells, the fluorescence was located in an area near the nucleus, as opposed to uniform staining of cell cytoplasm in normal prolactin cells (Figure 6). Occasional positive cells with anti-hGH or anti$^{17-39}$ACTH were also found, often in the periphery of the tumor. These cells were considered normal included cells.

Four prolactinomas were studied by electron microscopy. The tumors consisted of oval or polyhedral cells, each possessing a large nucleus with a prominent nucleolus. In 3 cases, the larger part of the cytoplasm was occupied by an extensively developed rough endoplasmic reticulum (RER), seen either in the form of parallel cisternae or as "Nebenkern." In one case, the RER was not developed, but free ribosomes were very numerous. Apart from this case, the Golgi complex was extensively developed and contained immature pleomorphic secretory granules. The number of spherical or pleomorphic secretory granules varied from tumor to tumor and also from cell to cell within the same adenoma. Only one adenoma (Case 14) appeared to be well granulated, with granule size ranging from 150 to 700 nm in diameter. Two tumors were sparsely granulated, and one (Case 20) was very sparsely granulated (Figure 7). Their secretory granules were smaller, measuring 150–450 nm in diameter. In one case, a misplaced exocytosis was observed, ie, the extrusion of secretory granules along the lateral cell membranes into the intercellular space. In one hemorrhagic adenoma, a large number of cells containing large pigment granules was evident. Most tumor cells possessed only a modest number of rod-shaped mitochondria with regular lamellar cristae, but in Case 20 numerous mitochondria showed a honeycomb pattern. Blood-filled spaces, not lined by endothelium, were present in all the tumors. The prolactin cells lining these spaces showed pseudopods or

Figure 3—Hyperplasia of various hyperactive cells with normal cordon pattern and numerous capillaries. Case 27. (Masson's trichrome, ×640) (Compare with Figure 4) **Figure 4**—Prolactinoma. The small agranular cells are arranged in solid sheets and show signs of activity (large nuclei and nucleoli). Blood-filled spaces are never lined by endothelial cells. Case 48. (Herlant's tetrachrome, ×640) **Figure 5**—Prolactinoma with large cells (*right*) and normal pituitary (*left*). No connective capsule is observed. Case 18. (PAS-orange G, ×640) **Figure 6**—Immunofluorescence with anti-rPRL serum. Normal prolactin cells (*upper*) where the fluorescence is uniform and tumoral prolactin cells (*lower*) where the fluorescence is weaker and located near the nucleus. Case 41. (×400)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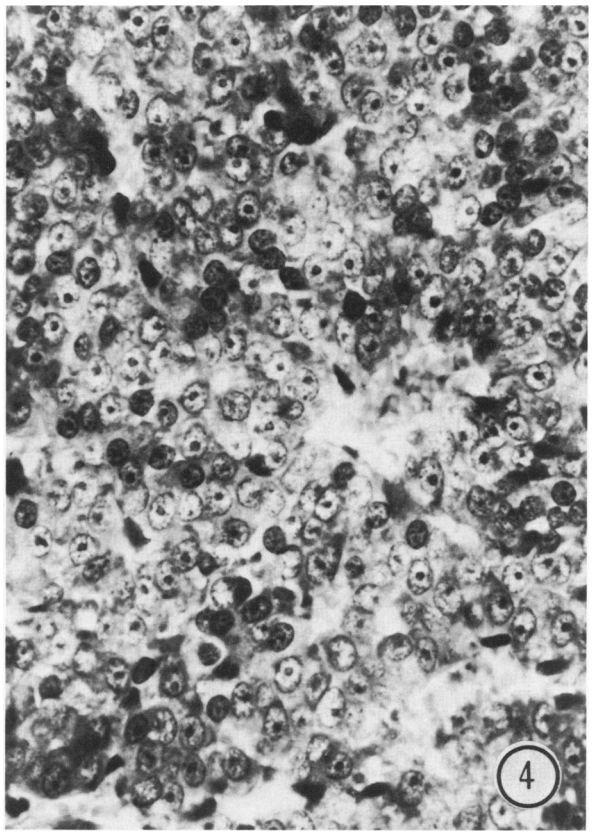
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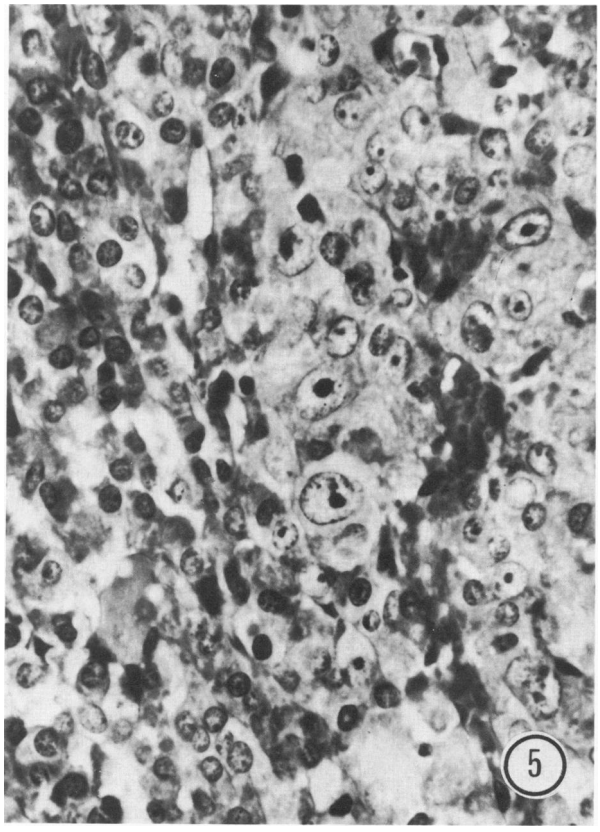
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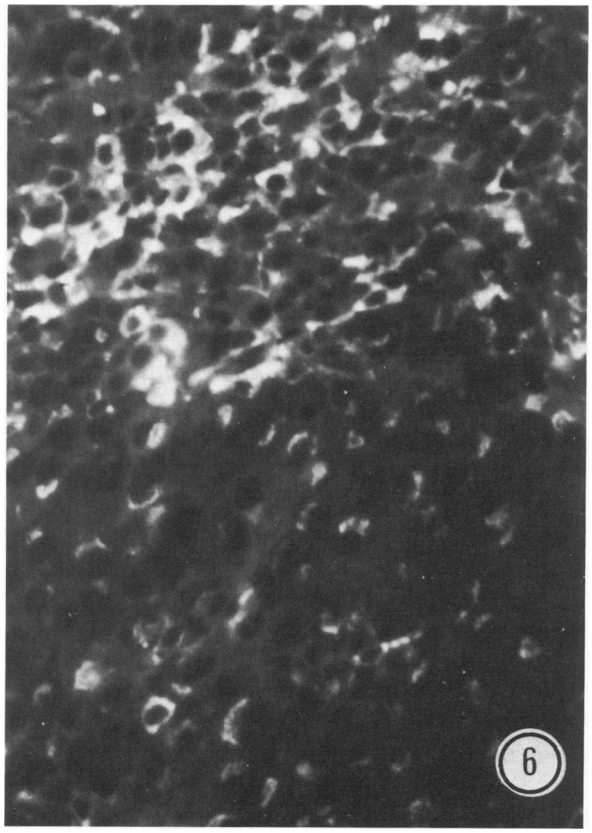
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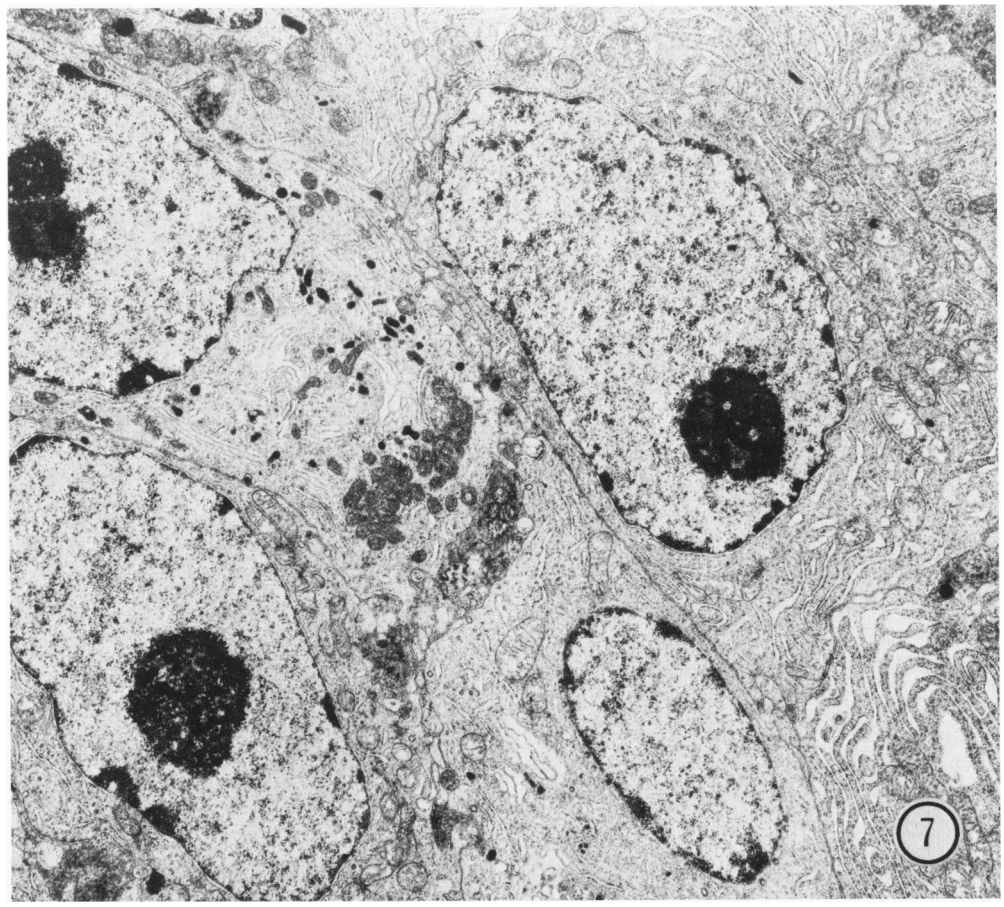


Figure 7—Very sparsely granulated prolactinoma with Golgi complex containing immature pleomorphic secretory granules and developed RER. Case 20. (×12,000)

microvilli. In Case 14, the extracellular spaces were enlarged, and numerous irregular microvilli were also seen. In general, the capillaries failed to show marked abnormalities, and the disruption of vascular walls was not seen.

In the *nonimmunostaining adenomas*, the capillaries were numerous, without blood-filled spaces and hemorrhage. The cells were arranged in cords (Figure 8). Their cytoplasm was fairly abundant, blue, PAS-negative, agranular, and slightly vacuolated. Nuclear signs of cellular activity were seen; however, no hormone was detected by immunocytochemistry (Figure 9). These cells looked like spongiocytes and, as other investigators did, we called them "spongiocytic adenomas."

Associated Pathology

In 7 rats, there was a leukemia with an always marked splenomegaly, and with a moderate hepatomegaly (3 cases) and, in 1 case, a generalized lymphadenopathy. Five of these 7 leukemias were associated with a pituitary tumor. Liver and spleen were usually flooded with the leukemic cells. Three pituitary tumors were infiltrated by leukemic cells.

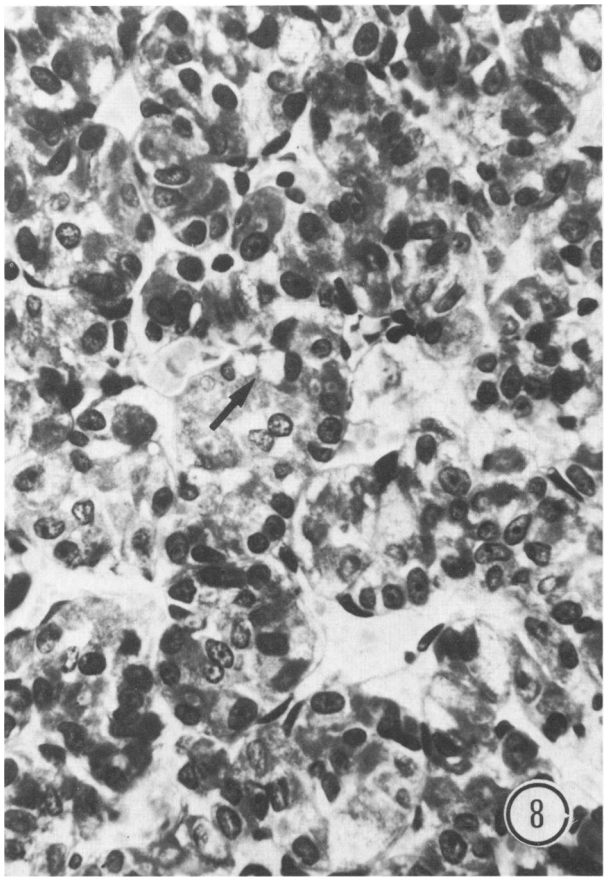
Two types of leukemia were found: lymphoid leukemia and mononuclear-cell leukemia, where the leukemic cells were characterized by an oval or round nucleus and a cytoplasm containing striking reddish granules.

Six rats had miscellaneous tumors. One sweat gland adenoma, one salivary gland adenoma, and two malignant tumors (one mammary carcinoma and one leiomyosarcoma) were associated with pituitary tumors. A Leydig cell tumor and an adrenal cortical tumor were found in 2 males without an accompanying pituitary adenoma.

The 2 rats that had the largest prolactinomas were noted to have a mammary hyperplasia, and a testicular and adrenal atrophy, respectively.

Prolactin RIA and Relationship With Pituitary Findings

In the rats without a pituitary adenoma, the mean plasma PRL value was 15 ± 1.8 μg/l (range, 6–36.5



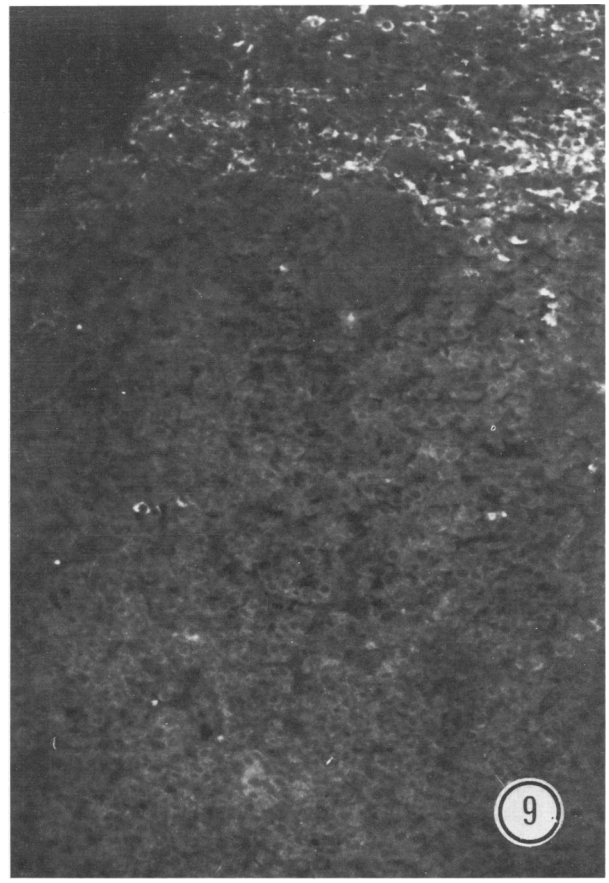


Figure 8—Spongiocytic adenoma. The cells are arranged in cords, and their abundant cytoplasm is slightly vacuolated (*arrow*). Case 9. (Herlant's tetrachrome, ×640) **Figure 9**—Spongiocytic nonimmunostaining adenoma. Immunofluorescence with anti-rPRL serum. At the upper part, normal positive prolactin cells and, at the lower part, tumoral negative cells. Case 23. (×160)

μg/l). There was no significant difference between the prolactinemia in males and females (14.8 ± 2 μg/l, and 15.2 ± 3.8 μg/l, respectively). The prolactinemia was normal in rats with a nonimmunostaining adenoma. In the 18 rats with a prolactinoma, the mean plasma PRL value was 213 ± 72.5 μg/l (range, 6–1050 μg/l). In 4 microprolactinoma-bearing rats, the plasma PRL value was normal, and at the upper limit in two others. Excluding Case 19 (the largest prolactinoma with the lowest prolactinemia), a linear correlation (r = 0.88) was found between the logarithm of the pituitary weight and the logarithm of the plasma PRL value (Figure 10), and between the logarithm of the tumor size and the logarithm of the prolactinemia (r = 0.84) (Figure 11). In the 4 rats where a circumscribed hyperplasia of hyperactive cells was noted, the prolactinemia was 6, 33, 36.5, and 42 μg/l, respectively. A value of 174 μg/l was found in a female rat without a pituitary adenoma, but its pituitary showed signs of cellular hyperactivity. A prolactinoma was implicated in every case where the prolactinemia was greater than 50 μg/l, with one exception.

Table 1 summarizes the main data concerning the pituitary-adenoma-bearing rats.

Incidence of Pituitary Adenomas and Major Diseases

Table 2 tabulates the incidence of the two kinds of pituitary tumors in relation to sex and age. The incidence of pituitary adenomas is 38% in rats older than 10 months. Almost all these adenomas are prolactinomas (86.4%); so the incidence of prolactinoma is 32.7% in the strain. The incidence of pituitary adenomas is higher in the females (55.5%) than in males (24.1%). This predominance in the female sex is more marked for the prolactinomas. Of 19 prolactinoma-bearing rats, 14 were females (74.2%), and at 28–32 months 8/9 females had a prolactinoma (88.8%). Apart from an uncommon prolactinoma, pituitary tumors occurred in animals over 17 months of age. The tumor and prolactinoma incidences rise

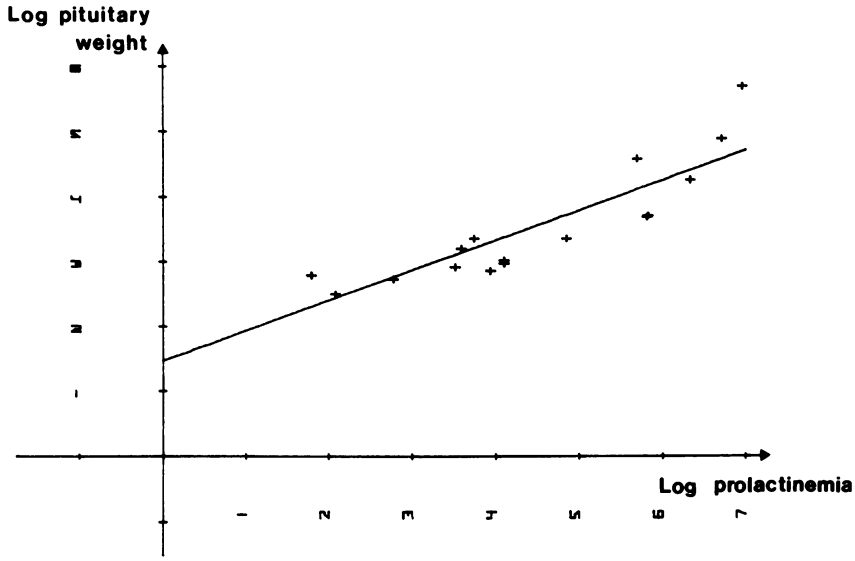


Figure 10—Prolactinoma. Linear correlation (r = 0.88) between the logarithm of the pituitary weight and the logarithm of the prolactinemia.

with age (at 10–12 months, 5.8%; at 17–18 months, 23%; at 24–25 months, 58.3% and 50%, respectively), with a maximum at 28–32 months (68.7% and 56.2%, respectively). The mean age of the pituitary tumor-bearing rats was 26 ± 1.35 months. Of the 12 microadenomas, 8 occurred between the ages of 17 and 25 months. At 30 months, 6 of 11 tumors were large. In comparison with the pituitary adenoma incidence, the leukemia and neoplasm incidence was low (12% and 10%, respectively).

Comparison of Rat Prolactinomas and Human Prolactinomas

In the 57 human prolactinomas of our series, the predominance in women was evident (36 women and 21 men). The female sex incidence (63.1%) in man is comparable to that found in the W/Fu/Ico rat strain. The mean age of the patients at the time of surgery was 38 years. The preoperative plasma PRL values varied from 35 to 26,200 μg/l, with an average of 1816 ± 4450 μg/l. Without precise tumor size evaluation, a tendency for larger tumors to be associated with higher plasma PRL levels may be suspected. From a cytologic point of view, the characteristics of the human prolactinomas were comparable to those of the rat prolactinomas. The cells were arranged in solid sheets, and hemorrhagic areas were noted in 33 cases. All the adenomatous cells were agranular in 39 cases (Figure 12a). Rare erythrosinophilic cells were scattered in 15 cases. Only one adenoma was densely granulated. The cellular size, the cytoplasmic baso-

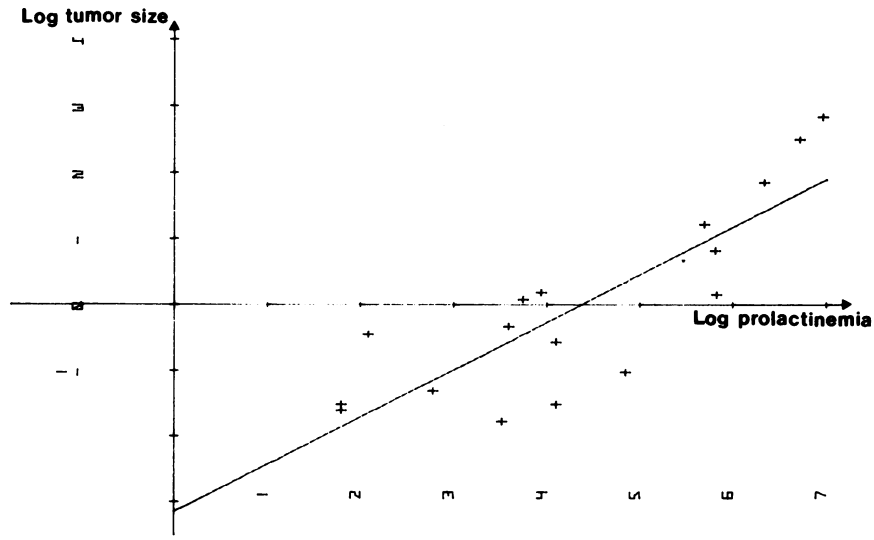


Figure 11—Prolactinoma. Linear correlation (r = 0.84) between the logarithm of the tumor size (sq mm) and the prolactinemia.

Table 1—Main Data on the Pituitary-Adenoma-Bearing W/Fu/Ico Strain of Rats

Rat	Age (months)	Sex	Plasma PRL (μg/l)	Pituitary findings: Wt (mg)	Pituitary findings: Macroscopic	Pituitary findings: Size (sq mm)	Pituitary findings: Microscopic	Associated diseases
19	11.5	M	<6	274	Invasive adenoma	28	Uncommon prolactinoma	Leukemia, testicular and adrenal atrophy
58	17	F	6	16	Normal	0.20	Microprolactinoma	–
54	17	F	6	16	Hemorrhagic spot	0.22	Microprolactinoma	–
56	17	F	60	20	Microadenoma	0.57	Microprolactinoma	–
37	25	M	8	12	Normal	0.64	Microprolactinoma	–
41	25	M	16	15	Normal	0.27	Microprolactinoma	–
						0.21	Microprolactinoma	–
40	25	F	33.5	18	Normal	0.17	Microprolactinoma	–
46	24.5	F	22.5	23	Macroadenoma	1.44	Spongiocytic adenoma	–
48	24	F	126.5	28	Normal	0.36	Microprolactinoma	–
50	24	F	331.5	39	Macroadenoma	2.26	Hemorrhagic prolactinoma	–
38	25	M	560	69	Macroadenoma	6.35	Hemorrhagic prolactinoma	–
9	32	M	12	11	Normal	0.25	Spongiocytic microadenoma	–
23*	28	M	·	14	Normal	0.80	Spongiocytic microadenoma	Leiomyosarcoma
24	29	F	60	19	Normal	0.22	Microprolactinoma	Sweat gland adenoma
21	32	M	51	17	Macroadenoma	1.20	Prolactinoma	Leukemia
22	28	F	36	24	Microadenoma	0.72	Microprolactinoma	–
28	30	F	42	28	Macroadenoma	1.08	Hemorrhagic adenoma	Leukemia
12	29	F	336	40	Macroadenoma	1.16	Hemorrhagic prolactinoma	Mammary carcinoma
10*	29	F	·	71	Macroadenoma	4.28	Prolactinoma	Leukemia, salivary gland adenoma
20	28	F	294	95	Macroadenoma	3.36	Hemorrhagic prolactinoma	–
14	28	F	819	129	Macroadenoma	12	Hemorrhagic prolactinoma	–
18	28	F	1050	289	Macroadenoma	16.8	Hemorrhagic prolactinoma	Mammary hyperplasia

* Died.

philia, and the nuclear signs of activity were variable but often marked. No connective tissue capsule was seen (Figure 12b). By immunofluorescence, almost all cells were positive with, an anti-hPRL antibody. However, in 4 cases, only 10% of the cells were immunostained. In one case, the positive cells were extremely rare. In most cases, as in the rat, the whole cytoplasm was positive; moreover, in 14 cases the fluorescence was located in a triangular area near the nucleus (Figure 13). The ultrastructural characteristics (rough endoplasmic reticulum, pleomorphic granules, large Golgi complex) were similar to those observed in the rat. However, the granule size was always smaller than in the prolactin cells of the rat (Figure 14). As in the rat, no histologic, immunocytochemical, or ultrastructural differences were observed between the large prolactinomas and the microadenomas.

In 15 cases, a fragment of nontumoral adenohypophysis was studied. No difference was found with the normal tissue. In no case was prolactin cell hyperplasia evident. The number of prolactin cells revealed by immunofluorescence varied from 0% to 30%.

Discussion

The incidence of spontaneous pituitary adenomas in the W/Fu/Ico strain is 38% in rats over 10 months and 53.6% in rats over 17 months. The predominance in females is marked (71.4% in the females and 35% in the males over 17 months). These results are very different from those reported by Kim et al (27.3% in 43 females between 17 and 30 months)[29] and by Ito et al (60% in 10 males over 20 months).[30] These discrepancies may perhaps be explained by an evolution of the strain or by errors due to the difficulty in distin-

Table 2—Incidence of Pituitary Tumors According to Histologic Type and Rat Age and Sex

Age (months)	Number of rats examined	Prolactinomas: Number of tumor-bearing rats	Prolactinomas: %	Spongiocytic adenomas: Number of tumor-bearing rats	Spongiocytic adenomas: %	Pituitary Tumor Incidence (%)
10-12	9 M	1	11	0	0	11
	8 F	0	0	0	0	0
17-32	20 M	4	20	2	10	35
	21 F	14	66.6	1	4.7	71.4
Total	58	19	32.7	3	5.2	38

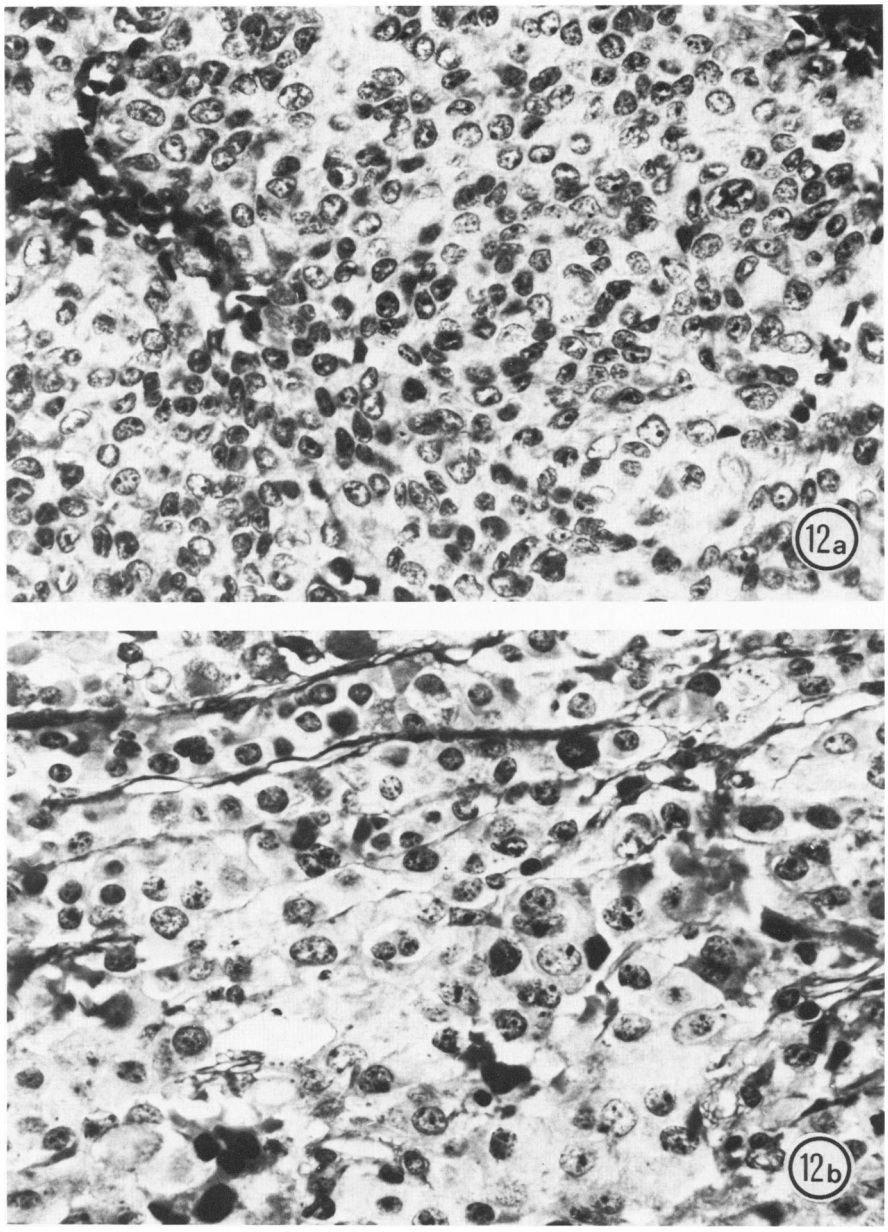


Figure 12—Human prolactinoma. **a**—The small agranular cells are arranged in solid sheets and show signs of activity (large nuclei with nucleoli). Two blood-filled spaces are observed. (Herlant's tetrachrome, ×640) (Compare with Figure 4) **b**—Prolactinoma with large cells (*lower*) and normal cordonal pituitary (*upper*). No connective capsule is observed. (PAS-orange G, ×640) (Compare with Figure 5)

guishing hyperplastic area from adenomas. Indeed, no clear-cut data have yet been given to differentiate between hyperplastic and neoplastic changes in the pituitary. We define microadenoma as a well-circumscribed nodular area found on numerous serial sections where the cordon pattern and normal cellular polymorphism have disappeared. A microadenoma may be observed in a gland with a regular surface and a normal weight. On the basis of our present studies, it is impossible to establish a relationship between a circumscribed hyperplasia of various hyperactive cells and a microadenoma. We can only point out that in 3 of the 4 cases with such findings the prolactinemia and the pituitary weight were at the upper limit of the normal. If we compare, for the same age, the incidence of spontaneous pituitary tumors in different rat strains studied by various investigators, we note that the Wistar, and especially the Wistar/Furth rat, has the highest incidence. Griesbach[35] and Ito et al[30] reported incidences of 94% and 70% in males of over 20 months in Long-Evans and Charles-River strains, but it would seem that hyperplastic areas were taken into consideration in these studies.

In the W/Fu/Ico strain, two types of pituitary adenomas were distinguished: nonimmunostaining spongiocytic adenoma and prolactinoma. The first type was described as early as 1948 and 1950 in various rat strains[8,10,12] and more recently in the Wistar strain.[24]

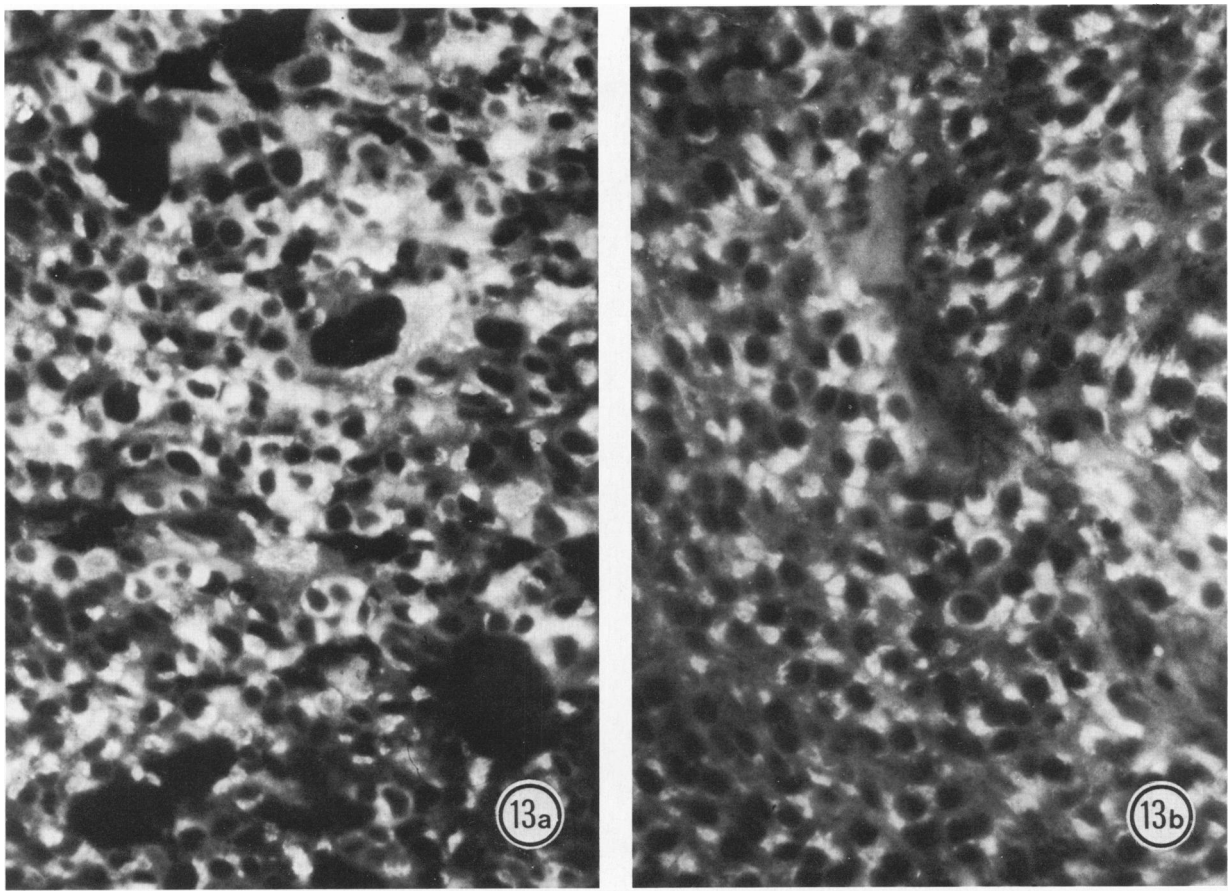


Figure 13—Immunofluorescence with anti-PRL serum. Comparison of rat prolactinoma (**a**) and human prolactinoma (**b**). (×400)

Spongiocytic adenoma is rare in the W/Fu/Ico strain (5.2%). It has never been described in man and thus presents less interest for the pathologist than the prolactinoma. Diagnosis of prolactin adenoma was made for the first time in the rat in 1950 by Oberling et al.[12] Our histologic and hormonal data are in agreement with previous observations.[20,22,24,25]

The prolactinoma of the W/Fu/Ico rat strain is often a hemorrhagic adenoma. The cells, often agranular and hyperactive, are in the majority positive with anti-rPRL antibody, have few polymorph granules, and have a well-developed rough endoplasmic reticulum. Plasma PRL concentrations were increased in most of the tumor-bearing animals (14 of 18). A mammary hyperplasia was associated with the highest value of plasma PRL. In our studies, the incidence of prolactinoma is 66.6% in females and 20% in males over 17 months. Recently, Prysor-Jones and Jenkins found a lower incidence in exbreeding females (60.0%) and in males (6.1%).[31] This discrepancy can be explained by the fact that they only took macroscopic tumors into account with a hyperprolactinemia >80 μg/l. Like Berkvens et al they reported somatotropic and prolactin adenoma. None was found in our series, nor in that of Kovacs et al.[22]

Histologic analogies and the different frequency according to age between micro- and macroadenomas prove that the microadenoma is not an anatomopathologic entity but rather a form of beginning. Moreover, as early as 1938, Wolfe et al had already established a similar relationship between "adenomatous nodule and hemorrhagic adenoma."[4] Certain microprolactinomas can be associated with a normal prolactinemia. In man, somatotropic, corticotropic, and gonadotropic silent adenomas were reported,[36-39] but no silent prolactinomas. In the rat, the existence of significant linear correlations between the size of the tumor, the weight of the pathologic pituitary, and the value of the plasma PRL makes it possible, according to the evolution of the prolactinemia, for one to evaluate the growth of the tumor and to study the antitumoral effects of certain drugs such as 2Br-α-ergocryptine (Trouillas et al, in preparation). We have proven in the rat, as is suspected in the human, that a hyperprolactinemia, even if considerable, is not nec-

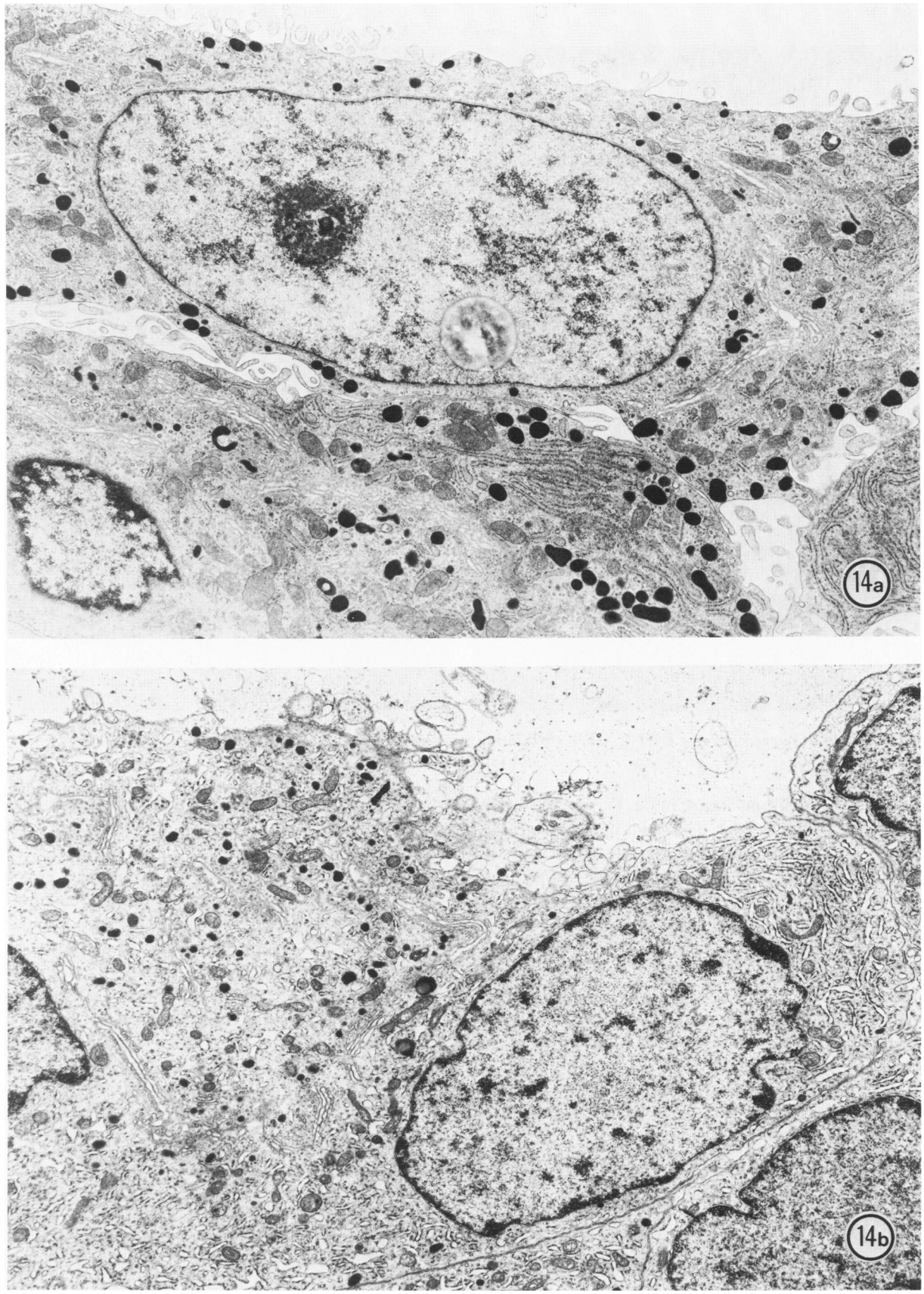


Figure 14 – Sparsely granulated prolactinoma. Comparison of rat prolactinoma (**a**) with enlargement of intercellular spaces, microvilli near a blood-filled space, pleomorphic secretory granules, developed RER and Golgi complex (× 15,000) and human prolactinoma (**b**), where the secretory granules are smaller than in rat tumoral prolactin cells. (× 12,000)

essarily tumoral in origin. This eventuality is extremely rare in the rat, which enables us to suspect a prolactinoma if the prolactinemia is greater than 80 μg/l.

We consider the rat of the W/Fu/Ico strain a good animal model for the study of the human prolactinoma for the following reasons: 1) the histologic, immunocytochemical, and ultrastructural analogies between prolactinomas in rats of the W/Fu/Ico strain and prolactinomas in the human;[40-51] 2) the predominance in the female sex in both species; 3) the relationship between the tumor size and the prolactinemia also observed in the human species;[52-56] 4) the high incidence of prolactinoma, coupled with the low incidence of associated disease in both species. The factors of human pituitary tumorigenesis remain unknown. The study of an animal model with spontaneous tumor, in our opinion, should not be neglected.

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