Effects of Bromocriptine on Prolactin-Secreting Pituitary Adenomas

Mechanism of Reduction in Tumor Size Evaluated by Light and Electron Microscopic, Immunohistochemical, and Morphometric Analysis

HIROSHI MORI, MD,* SHINTARO MORI, MD,† YOUICHI SAITOH, MD,‡ NORIO ARITA, MD,‡ TOSHIHIRO AONO, MD,§ TOHRU UOZUMI, MD,‖ HEITARO MOGAMI, MD,‡ AND KEISHI MATSUMOTO, MD*

Prolactin-secreting pituitary adenomas were studied to clarify the mechanism by which bromocriptine reduces tumor size. Patients examined consisted of three groups: Group I (four cases) received no medication, Group II (six cases) continued bromocriptine treatment (10 mg/day for 2 weeks) until the operation, and Group III (five cases) discontinued the treatment 1 week before the operation. Adenomas in Group II showed a variety of degenerative and necrotic changes of tumor cells in addition to marked decrease in volume of individual cell. Adenomas in Group III showed divergent structural changes. Irreversible changes seen in Group II became more pronounced with a marked increase in stromal tissue. Proliferative areas consisting of intermediate-sized cells were found in the scarce stromal tissue. The findings seem to indicate that the reduction in size of prolactinomas by bromocriptine treatment results from the reduction in size of individual tumor cell as well as from cell loss secondary to necrosis.
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BROMOCRIPTINE has been used extensively in the treatment for hyperprolactinemia irrespective of the cause, including prolactin-secreting pituitary adenomas (prolactinomas). This agent not only lowers prolactin (PRL) levels but also reduces tumor size.[1,2] However, the action mechanism of bromocriptine in reducing tumor size has not been established. Most tumors decrease in size and maintain their reduced size during bromocriptine treatment. The reduced tumors enlarge rapidly after termination of bromocriptine therapy. The reduction in tumor size has generally been considered to result from reversible reduction in size of individual tumor cell.[3–5] Some tumors, however, have been shown to maintain their reduced size even for 2 years after withdrawal of bromocriptine therapy.[6] A case of macroprolactinoma suggesting complete regression after 26 months of bromocriptine therapy was reported.[7] Recently, a "cytocidal" effect of bromocriptine on a prolactinoma has been reported by Gen *et al.*,[8] who observed a pronounced decrease in number and size of tumor cells with an increase in amount of stromal tissue.

The current study clarifies the mechanism by which bromocriptine reduces prolactinomas in size. To clarify whether the reduction in tumor size results exclusively from reduction in size of individual tumor cell, or whether cell loss due to necrosis is also responsible for reduction in tumor size, prolactinomas from 15 patients were studied by light and electron microscopic examination, immunohistochemistry, and by morphometry at light microscope level. We observed marked degenerative and necrotic changes with fibrosis of the tumor tissue, as well as reduction in size of individual tumor cell in prolactinomas treated with bromocriptine. Breakdown of tumor cells was clearly shown in all tumors examined in the current study.

Materials and Methods

The patients consisted of three groups (Table 1). Group I included four patients who received no medical therapy before adenomectomy and served for control group. Group II consisted of six patients who were treated with bromocriptine (10 mg/day) for 2 weeks.

From the Departments of *Pathology, ‡Neurosurgery, and §Gynecology and Obstetrics, Medical School, Osaka University, Osaka 530 Japan; †Department of Neurosurgery, The Center for Adult Diseases, Osaka 537 Japan; and ‖Department of Neurosurgery, Hiroshima University School of Medicine, Hiroshima 734 Japan.

Address for reprints: Hiroshi Mori, MD, Department of Pathology, Medical School, Osaka University, 3-57, Nakanoshima 4, Kita-ku, Osaka 530 Japan.

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TABLE 1. Clinical, Biochemical, and Radiologic Findings in Patients With Prolactinomas

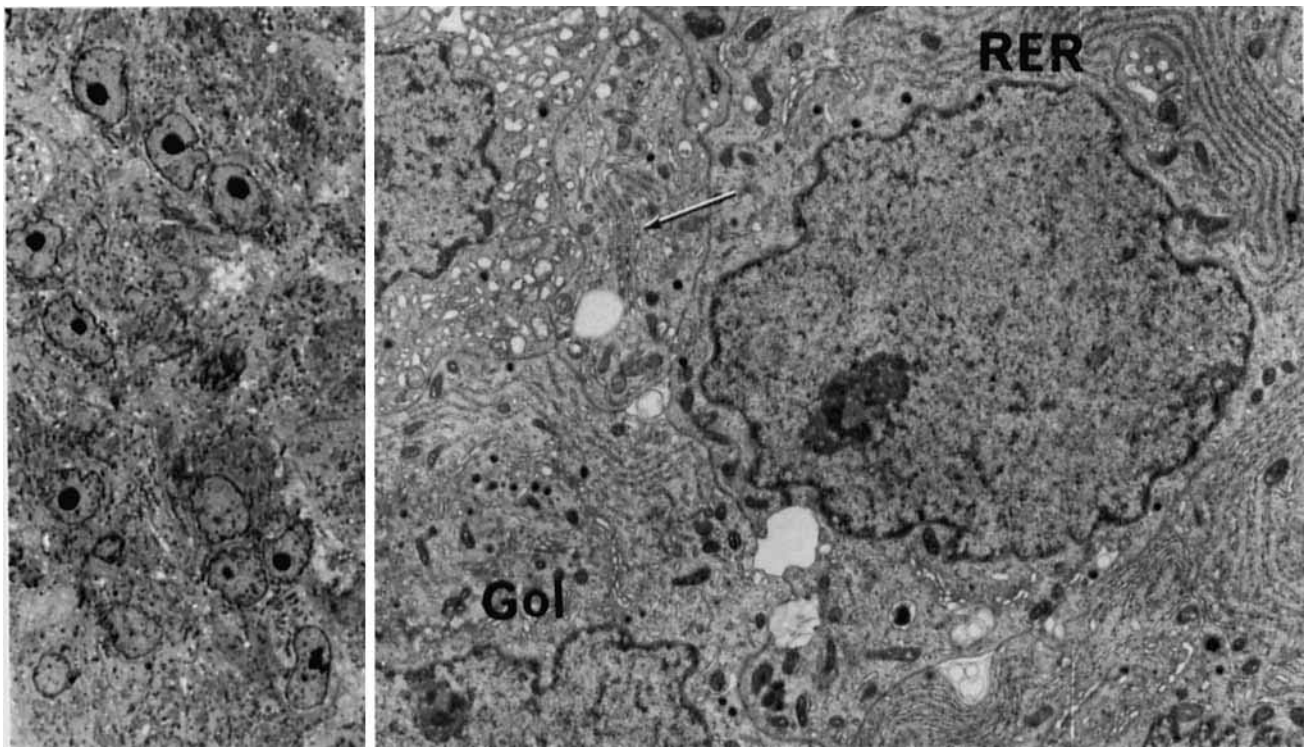
Group	Patient no.	Age/sex	Clinical symptoms (duration: yr or mo)	Serum PRL (ng/ml)			Tumor size* (diameter: mm)		
				Before	On CB	Off CB		Before	On or Off CB
I	1	24/F	Amenorrhea, galactorrhea (4 yr)	114	—	—	Micro	4	—
	2	28/F	Amenorrhea, galactorrhea (5 yr)	152	—	—	Expansive	12	—
	3	24/F	Amenorrhea, galactorrhea (3 yr)	300	—	—	Expansive	12	—
	4	19/M	Visual disturbance (6 mo), decreased libido (3 mo)	1450	—	—	Invasive	32	—
II	5	33/F	Amenorrhea, galactorrhea (11 yr)	62	12	—	Micro	3	Unchanged
	6	26/F	Amenorrhea, galactorrhea (4 yr)	721	10	—	Micro	9	NA
	7	24/F	Amenorrhea, galactorrhea (5 yr)	268	5	—	Expansive	12	Unchanged
	8	25/F	Amenorrhea, galactorrhea (6 yr)	1480	62	—	Expansive	14	11
	9	58/M	Decreased libido, galactorrhea (24 yr)	12,000	100	—	Invasive	31	NA
	10	50/M	Decreased libido (13 yr), visual disturbance (4 yr), nausea, vomiting (2 yr)	11,040	97	—	Invasive	48	Unchanged
III	11	30/F	Amenorrhea, galactorrhea (7 yr)	380	61	119	Micro	6	Unchanged
	12	33/F	Amenorrhea, galactorrhea (5 yr)	53	6	42	Micro	4	Unchanged
	13	32/F	Amenorrhea, galactorrhea (12 yr)	2145	45	764	Expansive	16	13
	14	40/F	Amenorrhea, galactorrhea (20 yr)	4190	87	3514	Invasive	34	Unchanged
	15	48/M	Decreased libido (2 yr), headache (7 mo)	4500	NA	770	Invasive	36	NA

* Micro: microadenoma (less than 10 mm in diameter); Expansive: macroadenoma (more than 10 mm in diameter) confined within sella turcica; Invasive: macroadenoma invading surrounding tissue.
CB: bromocriptine; NA: not available.

Group III consisted of five patients who underwent adenomectomy 1 week after cessation of the bromocriptine treatment for 2 weeks.

Serum PRL levels were measured by radioimmunoassay using homologous human PRL with an interassay variation of 10% at 5 ng/ml. At our institution, the normal values for PRL are less than 25 ng/ml. Tumor size was estimated from a diameter determined on computerized tomography (CT) scan in macroadenomas, and also from an amount of tissue fragments removed by surgery in microadenomas. Tumor tissues excised were fixed either with 10% formalin buffered with phosphate for light microscopic study, or with 3% glutaraldehyde buffered with *s*-collidine for electron microscopic study. Immunohistochemical staining for PRL was performed on paraffin sections by immunoperoxidase method. Electron micrographs were taken on thin sections cut from epon blocks, contrasted with uranyl acetate and lead citrate using a Hitachi 12 electron microscope at 100 kV.

Morphometric analysis was performed on 0.5 μm-thick epon sections stained with toluidine blue, using a point-counting method at light microscopic level. Morphometric procedures used in the current study were almost the same as detailed previously.[9,10] In brief, 5 to 8 representative sections were chosen from 10 to 20 blocks processed for electron microscopic examination in each tumor. Since specimens in Group III consisted of areas showing two different histologic features, sections examined were chosen in proportion to the number of blocks showing each feature. Sections were viewed at magnification of $\times$1000 through an eyepiece equipped with a lattice grid containing 100 test points in area equal to 1 cm^2. Volume density of the stromal tissue was estimated by counting hit points on the stromal tissue. Volume of nucleus and cytoplasm of an individual



FIGS. 1A AND 1B. Group I (control). Light micrograph cut from epon block, stained with toluidine blue (A, left, ×930), and electron micrograph (B, right, ×7500), respectively. Tumor cells have a nucleus with a prominent nucleolus and abundant cytoplasm, in which rough ER (RER) and Golgi apparatus (Gol) are well developed. An arrow indicates annulate lamellae.

tumor cell was obtained by dividing volume density of each structure by the number of tumor cells within unit volume of tumor tissue (numerical density). The volume of an individual tumor cell was a sum of that of the nucleus and the cytoplasm. The numerical density was estimated with the number of tumor cell nuclei counted within test areas and a mean diameter of nucleus using a formula of Floderus.[9] The number of fields examined was 70 to 140 in total per tumor, and number of nuclei measured was approximately 300 per tumor.

Results

Serum PRL Levels and Tumor Size

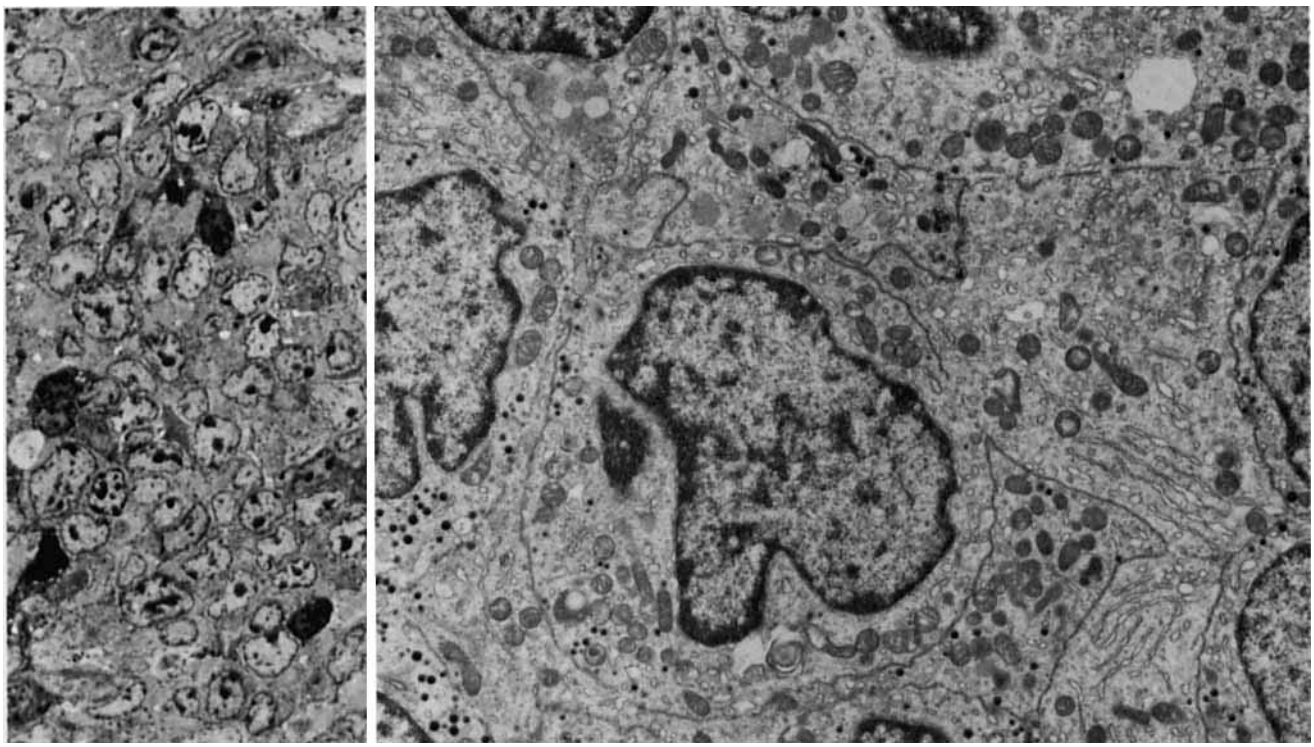
All patients examined in Groups II and III showed a pronounced decrease in serum PRL levels after the bromocriptine treatment (Table 1). The PRL levels in patients in Group III rose 1 week after withdrawal of bromocriptine, but the levels were still lower than those before bromocriptine. Tumor size was measured on CT scan in 8 of 11 patients during bromocriptine treatment or after its withdrawal. There were two expansive macroadenomas that showed evident reduction in tumor size. Other tumors failed to show reduction in tumor size.

Light and Electron Microscopic Examination

In control group (Figs. 1A and 1B), tumor cells formed solid cell nests separated by a small amount of stromal tissue. There was neither definite necrosis nor fibrosis. Electron micrographs showed that abundant cytoplasm contained well-developed rough endoplasmic reticulum (ER) and Golgi apparatus, as reported previously.[11,12] The tumor cells were mostly sparsely granulated. Misplaced exocytosis was occasionally seen.

Tumors treated with bromocriptine for 2 weeks (Group II) showed a variety of structural changes. They included reduction in cell size, degenerative and necrotic changes of the tumor cells, and increase in amount of stromal tissue. Although these alterations varied in extent from tumor to tumor, they were found in all six tumors examined.

First, tumor cells became noticeably smaller in size (Figs. 2A and 2B). The nucleus was irregular in contour and had clumped chromatin. Rough ER and Golgi apparatus reduced remarkably. Secretory granules increased in number. Some tumors had moderate number of uniform-sized, smaller granules (approximately 200 nm in diameter, Figs. 2A and 2B), whereas other tumors had exceptionally numerous granules varying in size from 140 to 800 nm (Figs. 5A and 5B).



FIGS. 2A AND 2B. Group II (ON bromocriptine therapy). Tumor cells are small in size and have an irregular-shaped nucleus. Rough ER and Golgi apparatus are poorly developed. Secretory granules increase in number (A, left, ×930; B, right, ×7500).

The second change was degeneration of the tumor cells. The cytoplasm contained numerous vacuoles, presumably fragmented rough ER, and a considerable number of lysosomes and lipofuscin granules. Mitochondria underwent either swelling or shrinkage. In some tumor cells, most part of the cytoplasm was occupied by closely packed bundles of filaments (Fig. 3).

Necrosis or breakdown of the tumor cells was the third change. Single-cell necrosis was small in number and was scattered in the tumor nests (Fig. 4), whereas breakdown of tumor cells with cytoplasmic fragmentation occurred in clusters predominantly in the periphery of tumor nests (Figs. 5A and 5B, 6A and 6B). The plasma membrane of the latter cells was indiscernible. Collagen fibers appeared to invade tumor cytoplasm and were intermingled with degenerated tumor cell organelles (Figs. 5A and 5B, 6A and 6B).

Spheroid bodies consisting of collagen fibers and membranous structures including secretory granules, probably derived from destructed tumor cells, were formed in the increased stromal tissue (Figs. 6A and 6B). Macrophages accumulated around these destructive areas and showed intense phagocytic activity. Secretory granules were taken up by the macrophages. Increase in amount of stromal tissue also was common. Collagen

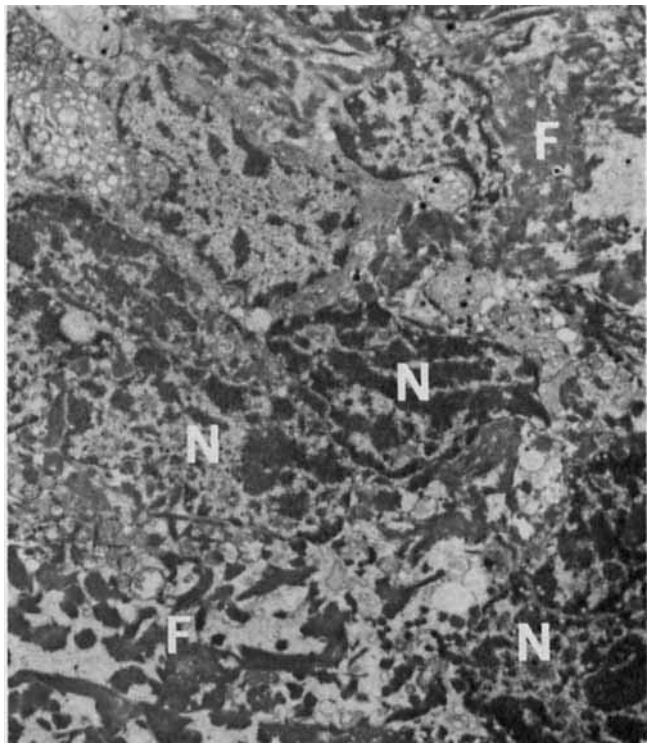


FIG. 3. Group II. Numerous bundles of filaments (F) are present in the cytoplasm of degenerating tumor cells, of which nuclei (N) are irregular in shape and have clumped chromatin (original magnification ×5200).

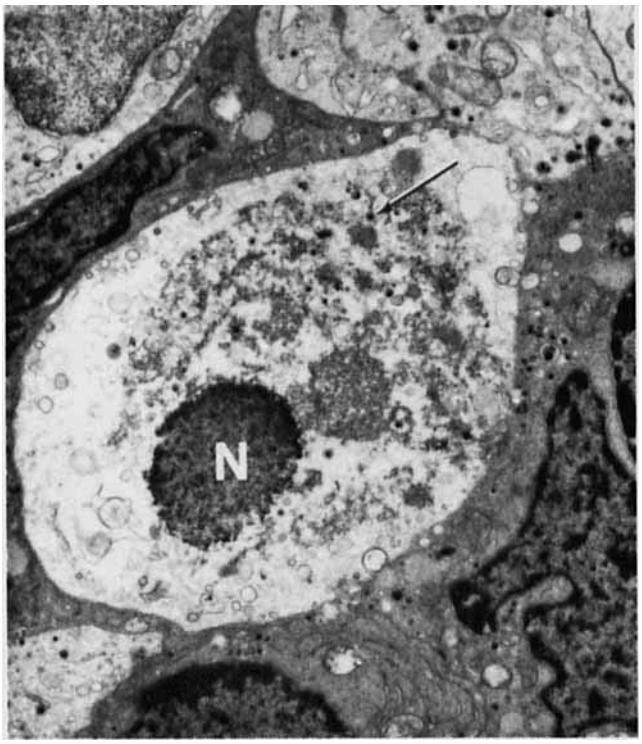


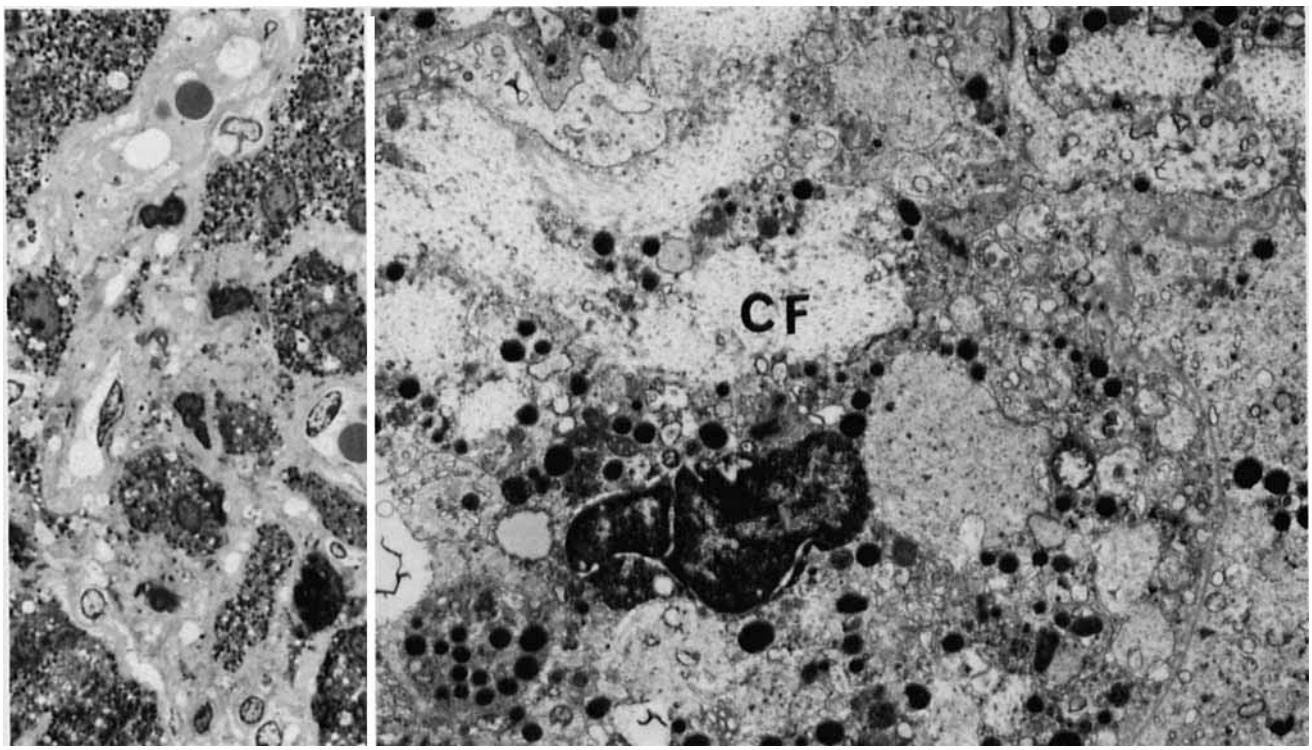
FIG. 4. Group II. Single-cell necrosis is scattered in the tumor nests. The nucleus (N) is pyknotic. Cytoplasmic organelles other than secretory granules (arrow) are not identifiable (original magnification ×8300).

fibers increased not only around blood vessels, *i.e.*, around the tumor nests but also within the tumor nests.

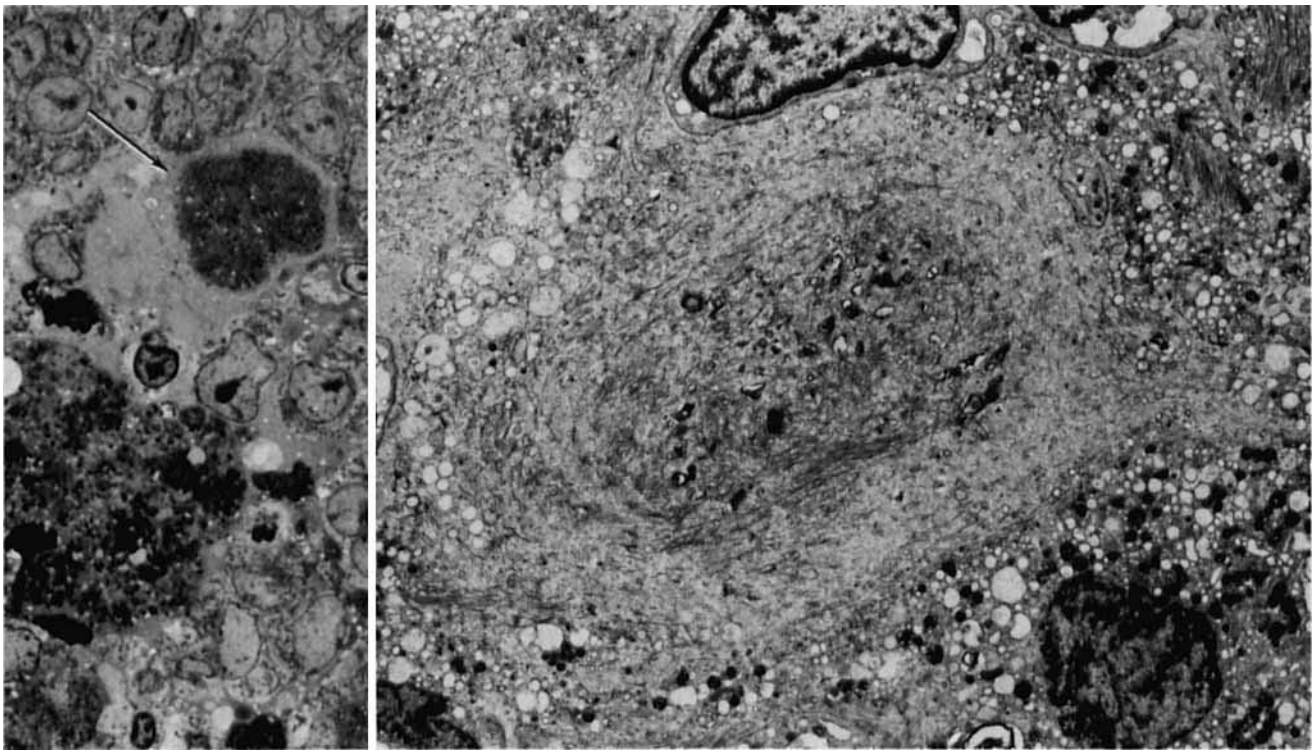
In Group III, tumors showed two distinct, divergent histologic appearances (Fig. 7). The one was a destructive change as seen in Group II, and the other appeared to be regrowth of tumor cells. The former was more advanced than in Group II. The stromal connective tissue increased much more in volume, and spheroid aggregations increased in number. In other areas, however, seemingly regrowing change was observed. Tumor cells which appeared to be similar to those of Group I, except for somewhat smaller size, occurred singly or in small clusters among degenerated and shrunken tumor cells in some tumors, or occupied most parts of other tumors. The cytoplasm contained moderately developed Golgi apparatus and rough ER. A few secretory granules were observed around Golgi apparatus. The stromal tissue was scanty in these areas.

Immunohistochemical Findings

Prolactin was localized in most tumor cells of Group I, though staining intensity varied from cell to cell. Tumor cells in Group II generally stained more intensely, compared to Group I. In the degenerating area in Group III, staining intensity was intermediate between Group



FIGS. 5A AND 5B. Group II. Tumor cells in the periphery of tumor nests undergo breakdown with collagen fibers invading tumor cell cytoplasm (CF). The plasma membrane is discernible. This adenoma has numerous secretory granules up to 800 nm in diameter (A, left, ×930; B, right, ×7500).



FIGS. 6A AND 6B. Group II. (A, left) Light micrograph shows a spheroid body (arrow) and a cluster of necrotic tumor cells (lower left) (×930). (B, right) Electron micrograph shows the spheroid body to consist of collagen fibers and cell debris. The plasma membrane of the surrounding tumor cells is invisible (×4300).

I and Group II, whereas cells in the regrowing area stained less intensely than in Group I.

Morphometric Findings

Volume percentage of stromal tissue within the tumor (stromal volume density) was larger, and volume of individual tumor cell, particularly the cytoplasmic volume, was remarkably smaller in Group II as compared with Group I (Table 2). In the degenerative area in Group III (Group IIIa), stromal volume density was much larger than that in Group I or Group II. Tumor cells in Group IIIa had almost the same volume as Group II. Regrowing area in Group III (Group IIIb) had much smaller volume density of the stroma than in Group II or Group IIIa, and larger volume of individual tumor cell than in Group II. Extent of increase in stromal volume and decrease in individual cell volume of microadenomas in Groups II and IIIa was similar to that of expansive or invasive macroadenomas (individual data not shown).

Discussion

Most reports have documented that size reduction of prolactinomas by bromocriptine therapy occurred after 3 months or longer, whereas some tumors have been reported to decrease in size within 2 weeks.[13] Generally, it is for macroadenomas that the size reduction can be confirmed radiologically. No microadenoma has been reported to decrease in size after bromocriptine therapy, partly because of limitation in resolving power of roentgenograms. In the current study, tumors treated with bromocriptine consisted of four microadenomas, and three expansive and four invasive macroadenomas. There were two expansive macroadenomas that evidently showed radiologic size reduction after bromocriptine treatment for 2 weeks. Nevertheless, remarkable decrease in serum PRL levels and pronounced morphologic alterations were found in all tumors treated with bromocriptine.

In spite of numerous reports concerning the size reduction of prolactinomas after bromocriptine therapy, as judged by improvement of clinical symptoms such as visual disturbance, or roentgenograms of tumors,[2,3,5,14] there are few reports on the morphologic alterations in human prolactinomas. Earlier observations[15,16] described that most tumor cells appeared unchanged after bromocriptine treatment, although some tumor cells underwent mild degenerative alterations. Rengachary *et al.*,[3] Tindall *et al.*,[4] and Barrow *et al.*[5] measured size of individual

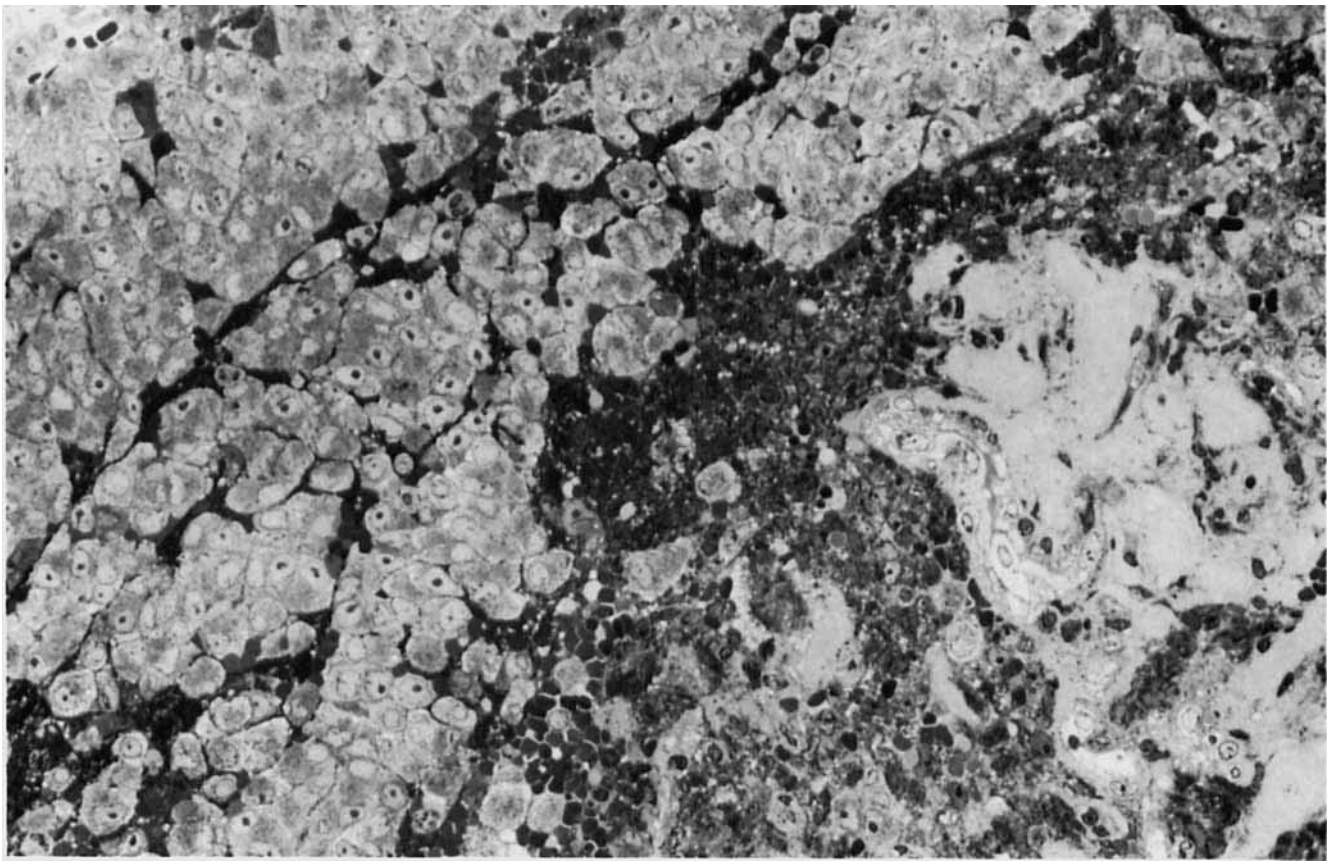


FIG. 7. Adenomas in Group III (OFF bromocriptine therapy) show degenerative and proliferative changes. The degenerative area (right half) shows fibrosis and shrinkage of adenoma cells with pyknotic nucleus and scanty cytoplasm, whereas cells in proliferative area (left half) have a pale, round nucleus with a prominent nucleolus and moderately abundant cytoplasm (×200).

tumor cells in prolactinomas treated with bromocriptine. Their morphometric analyses showed that bromocriptine treatment decreased individual tumor cell size with reduction in volume of organelles involved in PRL synthesis. The above investigators found neither cell necrosis, infarction, nor vascular injury, and consequently concluded that the reversible decrease in volume of prolactinomas is explained, at least in part, by the

TABLE 2. Morphometric Data on Changes of Prolactinomas Induced by Bromocriptine Therapy*

Group	Volume of stromal tissue within the tumor (%)	Volume of individual tumor cell		
		Nucleus (μm^3)	Cytoplasm (μm^3)	Whole cell (μm^3)
I Control (n = 4)	10.0 ± 1.4	370 ± 22	1,140 ± 81	1,510 ± 101
II On bromocriptine (n = 6)	23.3 ± 3.2†	269 ± 29†	636 ± 100†	905 ± 114†
III Off bromocriptine (n = 5)	27.2 ± 3.4†	294 ± 23	893 ± 83	1,187 ± 98
IIIa Degenerative area (n = 4)	43.4 ± 2.8†‡	241 ± 13†	781 ± 108†	1,022 ± 117†
IIIb Regrowing area (n = 4)	12.9 ± 2.4‡§	345 ± 17§	995 ± 110‡	1,340 ± 127‡

* Values are expressed as mean ± SEM.
† Significantly different ($P < 0.05$) from Group I.
‡ Significantly different ($P < 0.05$) from Group II.
§ Significantly different ($P < 0.05$) from Group IIIa.

reduction of cell volume and not by cell loss secondary to necrosis.

The current observations, however, have led us to a different conclusion: in addition to reduction of cell size, cell loss due to necrosis is responsible for the reduction of tumor size by bromocriptine treatment. Light and electron micrographs showed a variety of degenerative and necrotic changes. Although single-cell necrosis was small in number, breakdown of tumor cells with cytoplasmic fragmentations was frequently seen in clusters. The increase of stromal tissue with accumulation of collagen fibers after treatment with bromocriptine also was conspicuous in the current study. This may be interpreted as replacement fibrosis secondary to cell necrosis, when various degenerative and necrotic changes are taken into consideration. None of reports cited above described the fibrosis. Recently, Gen *et al.*[8] examined a prolactinoma which decreased remarkably in size after bromocriptine treatment for 8 months. They observed the tumor to consist of compact cell nests surrounded by ample stromal tissue. The current study showed for the first time the cell necrosis and replacement fibrosis during bromocriptine treatment in a moderate-sized group of prolactinomas.

It is of interest to what extent the size reduction of individual tumor cell and the cell loss, respectively, contributed to the reduction in tumor size. As shown in Table 2, it is obvious that bromocriptine decreased cell volume noticeably, particularly the cytoplasmic volume, and increased the stromal volume within the tumor. These effects of bromocriptine still continued during the 1 week after withdrawal of bromocriptine. On the other hand, it is not easy to evaluate how many tumor cells were lost by bromocriptine treatment. The number of total tumor cells is a product of tumor tissue volume and numerical density (number of tumor cells within a unit volume of tumor tissue). Measurement of an extent of the change in tumor tissue volume depends on a resolving power of CT scan.

In the current study, the change of total tumor cell number could be estimated in two expansive macroadenomas. In Case 8, in spite of an increase in numerical density to 180% of the pretreatment value after bromocriptine treatment (data not shown), the number of total tumor cells was estimated to be 90% of the pretreatment value because of a 50% reduction in tumor volume. The volume of the individual tumor cell decreased to 49% of the pretreatment value. On the other hand, the tumor of Case 13 reduced its volume to 54% and the numerical density to 86% (data not shown). Consequently, the number of total tumor cells decreased to 46% of the pretreatment value, whereas the volume of individual tumor cell was almost the same as that of control group 1 week after discontinuation of bromocriptine. These estimations indicate that tumor size reduction in Case 8 resulted predominantly from reduction in size of individual tumor cell, and the tumor size reduction in Case 13 was predominantly due to the reduction in number of total tumor cells. The extent to which tumor cell loss contributed to size reduction of bromocriptine-treated prolactinomas seemed to vary considerably from tumor to tumor.

Experimental observations indicate that bromocriptine decreases exocytosis of PRL,[17,18] presumably by lowering cyclic AMP levels.[19] Subsequently, intracellular PRL levels rise, mainly by increase in number of secretory granules, which in turn may inhibit DNA synthesis[20–22] and mitotic activity.[22–24] This action is reversible and may be called a "cytostatic" action.[8] However, mechanism of a "cytocidal" action as seen in the current study is not clear. This must be clarified in future study.

It seems, therefore, that there are two populations in prolactinoma cells, as proposed by Gen *et al.*[8] Most prolactinoma cells seem to be sensitive to "cytostatic" action of bromocriptine. Some tumor cells are sensitive to "cytocidal" action, and others are resistant. As shown in Figure 7, cells sensitive to "cytocidal" action undergo degeneration and necrosis, and resistant cells proliferate again after discontinuation of bromocriptine. The latter seems responsible for the regrowth of prolactinomas.

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