Pituitary Melanocorticotrophinoma with Amyloid Deposition

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SUMMARY: *The light and electron microscopic features of a pituitary adenoma composed of adrenocorticotrophic hormone (ACTH) and melanocyte stimulating hormone (MSH) cells with perivascular amyloid deposition is reported. Histochemical and fine structural data indicate that this material is APUDamyloid and is present in the extra-cellular perivascular spaces. It is suggested that the differences in fine structure and of distribution of the amyloid in pituitary adenomas is dependent upon the cell of origin.*

RÉSUMÉ: *Les aspects aux microscopes optiques et électroniques d'un adénome pituitaire composé de cellules ACTH-MSH, avec dépôt périvasculaire amyloide, sont détaillés. Les données histochimiques et de structure fine indiquent que ce matériel est APUDamyloide et est présent dans les espaces extracellulaires périvasculaires. Il est suggéré que les différences de structure fine et de distribution de l'amyloide dans les adénomes pituitaires sont dépendants de la cellule d'origine.*

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INTRODUCTION

Amyloid deposition in adenomas of the pituitary gland is not common (Pearse et al, 1972). We recently reported a prolactin producing pituitary adenoma (Bilbao et al, 1975) with intracellular and extracellular accumulation of a substance with the staining characteristics of APUD amyloid. In this paper we describe the histologic, histochemical and ultrastructural features of an ACTH-MSH secreting amphophil pituitary adenoma with amyloid deposition in a woman with Nelson's syndrome.

CASE REPORT

In 1957, at the age of 11, this white girl had the onset of menses with hypermenorrhea. Thereafter she developed obesity, acne, hypertension and striae in the flanks. In 1959 laboratory investigations showed: a diabetic type of glucose tolerance test, 22.4 and 111 mgs/24 hrs. urinary hydroxycorticosteroids before and after ACTH stimulation and a normal skull x-ray. The entire right adrenal gland (8 grams) and part of the left (2.6 grams) were removed and found histologically normal. Postoperatively the blood pressure became normal. The urinary 17 hydroxycorticosteroids were 2 mg/24 hrs. and rose to 31 with ACTH stimulation. In 1960, hirsutism and a 30 lb. weight gain were noted. X-rays showed osteoporosis of the spine. Urinary 17 hydroxycorticosteroids were 53 mg/24 hrs. The remaining left adrenal gland was removed (7.5 grams with histological evidence of hyperplasia). Postoperatively treatment included cortisone acetate 25 mg and 9 alphafluorohydrocortisone 0.1 mg daily. Over the ensuing year she developed increasing pigmentation of skin and buccal mucosa, and in 1963 a bitemporal visual field defect was found and a pituitary tumor with suprasellar extension was selectively removed through a frontal craniotomy. Postoperatively she received 3,500 rads of cobalt 60 to her sella turcica. Shortly thereafter she complained of cold intolerance, constipation and amenorrhea. The PBI had fallen from 4.7 mcg. (before radiation) to 3.1 mcg. Similarly, her radioactive iodine fell from 15% to 8%. She was treated with 0.2 mgs of L-sodium thyroxine daily in addition to replacement cortisone. In 1970 she was described as darkly pigmented. She had lost 20 lbs. in weight over a short period of time. The PBI was 8.9 mcg., T3 resin sponge uptake 41% (on thyroxine 0.2 mg daily), radioactive iodine uptake was 43% and serum TSH was higher than 3.3 units/ml. Serum growth hormone was undetectable and immunoreactive ACTH in plasma was 220 pico grams per ml (upper normal 120 pg/ml). LATS was undetectable. Thyroxine was discontinued. By March 1971 T4 was 11.1 mgs and T3 38%. The PBI was 17 mcg., and radioactive iodine uptake was 44.2%. There was no cortisol response to hypoglycemia. The treatment consisted of carbemizole 15 mgs, sodium thyroxine 0.1 mgs, cortisone 25 mgs and 9 alphafluorohydrocortisone 0.2 mgs daily. Five months later the symptoms of hyperthyroidism had disappeared; however, radioactive iodine uptake after 6 days of 100 mcg. daily of cytomel was 65% indicating nonsuppressibility of the thyroid gland. In July 1973 RIA-beta MSH determination was 69.9 ng/ml.

In September 1974 she was readmitted because of a sudden onset of headache. An angiogram showed a suprasellar extension of the pituitary tumor which was removed by a transseptal transsphenoidal operation.

MATERIAL AND METHODS

Pieces of the tumors removed in 1963 and 1974 were fixed in 10% neutral formalin and embedded in paraffin. Sections 4-6 u thick were stained with the following techniques: Haematoxylin-phloxine-saffron, Orange G, Gomori's aldehyde fuchsin, PAS, Aldehyde thionin, Methyl violet, Toluidine blue, Crystal violet, Congo red, Thioflavine T and Tryptophan, Pearse (1968). Only the tumor resec-

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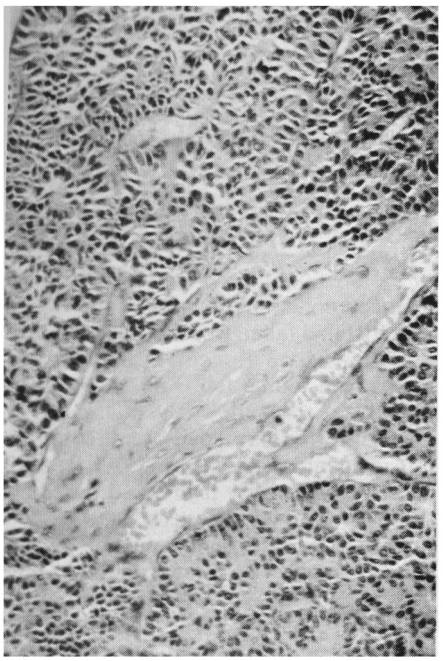
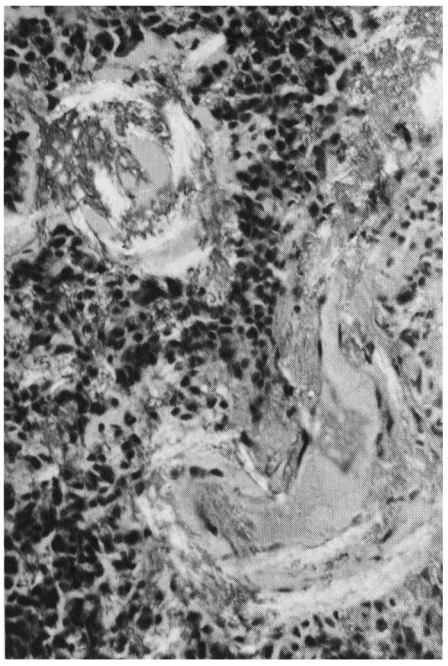


Figure 1—Pituitary adenoma removed in 1963. Polyhedral elongated cells orientated towards the blood vessels. Homogeneous substance in the perivascular spaces. Paraffin. Hematoxylin-phloxine-saffron. Original magnification x250.

Figure 2—Pituitary adenoma removed in 1974. Refractile material within vessel wall. Paraffin. Congo red. Photographed under half crossed polarizing filters. Original magnification x250.



ted in 1974 was available for electron microscopy. Pieces of tissue were fixed in 2.5 glutaraldehyde in 0.15 M Sorensen's buffer, post fixed in 1% osmium tetroxide in Millonig's bufer, dehydrated in graded ethanoland embedded in Epon 812. Thick sections were stained with Toluidine blue, the thin sections with uranyl acetate and lead citrate. They were examined with a Philips 300 electron microscope.

RESULTS

Light microscopic findings: The histologic features of the pituitary adenomas removed in 1963 and in 1974 were similar and will be described together. The tumor was composed of polyhedral, often elongated cells which appeared orientated toward the supporting blood vessels (Fig. 1). At some places an acidophil homogeneous substance coated the vessel wall and extended into neighboring areas enclosing few cellular elements. This material exhibited green yellow dichroism under polarized light, affinity for Congo red (Fig. 2), metachromasia with Crystal violet, fluorescence after Thioflavine T treatment and no staining with the Tryptophan reaction. The cytoplasm of the adenoma cells contained acidophil and basophil granules as demonstrated by the Phloxine and Orange G stains as well as Gomori's aldehyde fuchsin, PAS, and Aldehyde thionin methods. In the resin embedded toluidine stained tissue a marked variation in the density, and margination, of the cytoplasmic granules of individual cells was obvious (Fig. 3).

Electron microscopic findings: The tumor was composed of polyhedral or spherical cells. The cell nuclei were round or often irregular with eccentric clumping of chromatin. The RER consisting of parallel arrays was well developed in the cytoplasm of most cells with large numbers of ribosomes studded on their external aspects. Free ribosomes were abundant. Round or rod shaped mitochondria with transverse cristae were numerous. All adenoma cells possessed varying numbers of spherical or slightly irregular secretory granules scattered

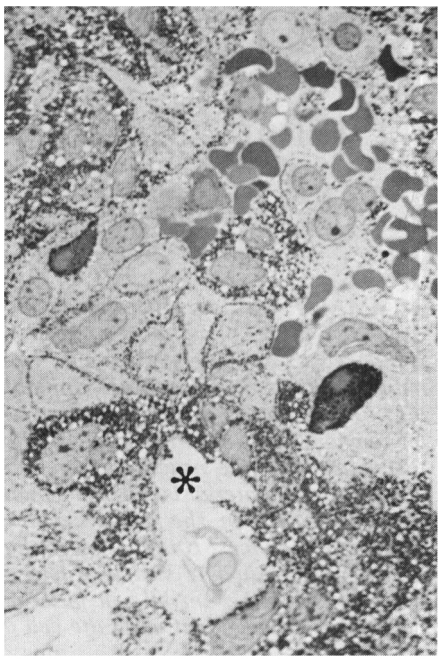
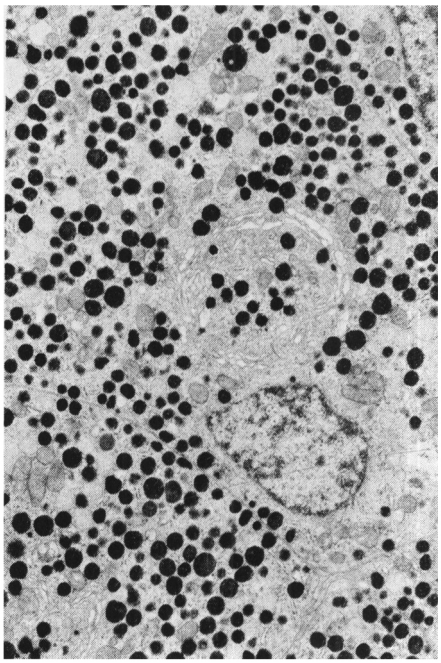


Figure 3—Homogeneous unstained substance in the perivascular spaces (asterisk). Marked variation in the granular content of the adenoma cells. Margination of secretory granules along cytoplasmic membrane. Epon embedded. Toluidine blue. Original magnification x1,000.

Figure 4—Densely granulated adenoma cells containing spherical or slightly irregular secretory granules and prominent Golgi zones. x7,700.



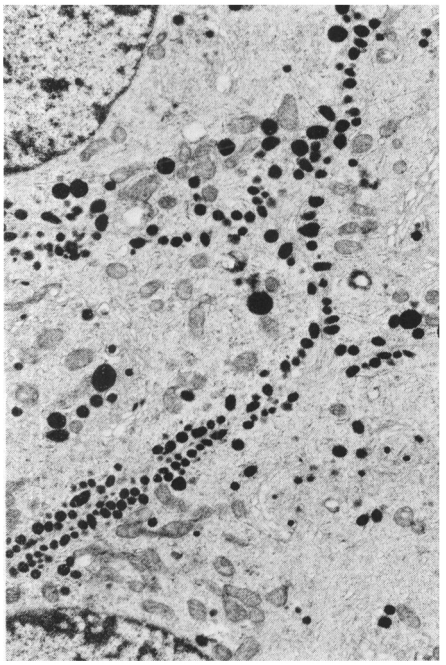


Figure 5—Margination of secretory granules is shown in a group of less densely granulated adenoma cells. x8,200.

uniformly in the cytoplasm (Fig. 4), or showing margination along the cell membrane (Fig. 5). Exocytosis was, however, not seen. The secretory granules ranged from 250 nm to 450 nm in diameter with an average of 350 nm. They varied considerably in electron density. The crescent or ring shaped Golgi apparatus was prominent with moderately dilated sacculi containing a few immature secretory granules (Fig. 6). A few cytoplasmic microtubules and microfilaments were also occasionally encountered. Large masses of filaments ranging from 75Å-95Å in diameter were noted adjacent to the capillaries, in the dilated subendothelial spaces (Fig. 7). They were never membrane bound, failed to show any periodicity and appeared closely apposed to adenoma cells occasionally mingled with collagen fibers. The location of the filamentous aggregates was carefully investigated. Though in some places a close spatial association was found between the filaments and the adenoma cells, no unequivocal evidence was obtained indicating intracellular accumulation of the filaments.

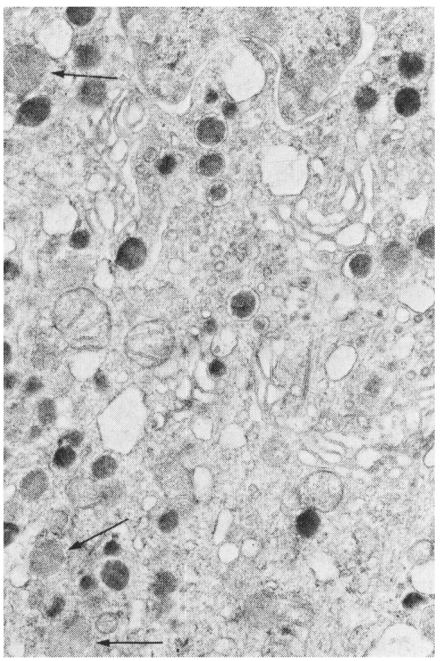
DISCUSSION

This case was regarded clinically as representing an ACTH-MSH secreting adenoma. By light microscopy it was found to consist of amphophil cells with cytoplasmic granular positivity for acid and basic dyes. Cushing's syndrome due to pituitary tumors whose cells exhibit mixed staining properties is not rare (Rovit and Berry, 1965). The ultrastructural appearance of the tumor conforms with the cases reported by Saeger (1973). Some variations in regard to size and distribution of secretory granules are possibly related to differences in functional activity.

The most unusual feature of this tumor was the presence of amyloid. Its location in the perivascular space associated with collagen fibers and its ultrastructure with a filament diameter ranging between 75Å and 95Å is reminiscent of immunoamyloid (Shirahama and Cohen, 1967). Histochemically, however, the negative reaction for tryptophan suggested that this material was endocrine amyloid or APUDamyloid (Pearse et al, 1972). This is in agreement with the concept that ACTH-MSH cells are part of the APUD system because of their ability to take up and decarboxylate amine precursors such as L-Dopa (Takor and Pearse, 1973; Pearse, 1974). Amyloid is a common finding in tumors derived from APUD cells of other locations such as medullary carcinoma of the thyroid gland (Ibanez, 1974). The ultra-structural differences between this present case and our previous report of amyloid in a pituitary adenoma (Bilbao et al, 1975) are remarkable. In the previous adenoma, which consisted of sparsely granulated prolactin cells the amyloid was composed of 150 Å wide tubules. It was RER bounded, partly intracellular, partly extracellular, and not related to blood vessels, but histochemically it appeared identical to that studied in the present case.

No explanation can be offered for the fact that the amyloid substance differed so markedly in fine structure and distribution in the ACTH-MSH cell adenoma, from that detected in the prolactin cell adenoma. It may well be that the amyloid was in a different phase of evolution and its fine structure and location de-

Figure 6—Well developed Golgi apparatus containing dense as well as relatively lucent (arrows) secretory granules. x18,000.



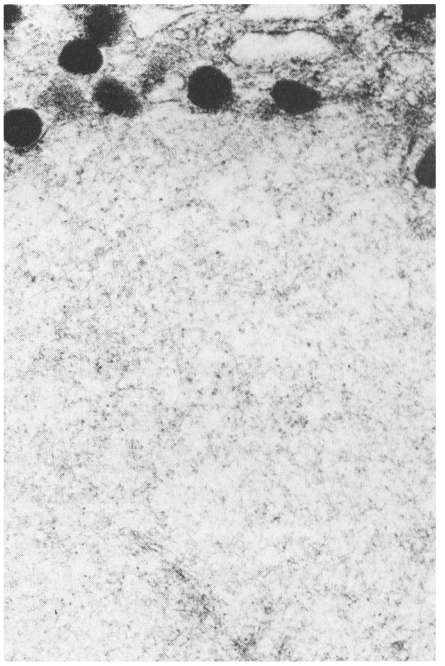


Figure 7—Clumps of amyloid fibrils adjacent to adenoma cells. x35,000.

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pended primarily upon its age. Alternatively, it is also possible that tumors arising from different pituitary cell types are accompanied by different amyloids. Careful study of further cases is required to resolve this question.

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