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Age-related Accumulation of Amyloid Inclusions in Adrenal Cortical Cells

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Cytoplasmic fine fibrillar inclusions with properties of amyloid occur as neurofibrillary tangles in the brain and in the aging choroid plexus. In the present study we show that inclusions, similar but not identical to those in the choroid plexus, are common in the adrenal cortex of elderly persons. The inclusions consist of aggregates of parallel fine fibrils, often in contact with lipid droplets and partially limited by a membrane. The inclusions have affinity for Congo red and exhibit a bright green birefringence after this staining. Therefore, the inclusions can be regarded as a form of senile amyloid. (Am J Pathol 1990, 136:461–466)

In the systemic amyloidoses, deposits of fibrillar proteins occur extracellularly in a variety of tissues. Several different proteins can give rise to the fibrils but each protein is characteristic of a certain amyloid syndrome. There is strong evidence that the molecular arrangement of the protein subunits is characteristic of the amyloid fibril and that this is responsible for the unique properties of them.[1] One such property of importance for the study of the diseases is green birefringence in polarized light after staining with Congo red.

Small depositions of a material, histologically and ultrastructurally similar to the amyloid in the systemic amyloidoses and occurring in certain tissues without any evidence of systemic disease, are more common. The brain and several polypeptide hormone-producing tissues as well as a few other tissues are especially prone to contain such localized deposits. Although these depositions usually are believed to be of no clinical importance *per se*, it is probable that they reflect a pathologic event in the affected tissue. Therefore, knowledge of the nature of the microdepositions could give insight both in the normal function of the tissue and in pathologic conditions.

In a pilot study of amyloid deposits in different endocrine tissues, we observed small deposits with amyloid properties within the cytoplasm of the cortex of the adrenal glands.[2] This prompted us to begin the present study, which shows that such deposits are very common and age related. Furthermore, we show that these inclusions have some resemblance to the deposits seen in the choroid plexus in older persons.

Materials and Methods

Patients

The adrenal glands were removed at autopsy from 114 randomly chosen patients. The material is schematically presented in Table 1.

Light Microscopy

Pieces of the adrenal glands and samples from the cerebrum, choroid plexus, left ventricle of the heart, lungs, and kidneys were fixed in 4% formaldehyde solution. They were embedded in paraffin, sectioned, and stained with van Gieson stain and alkaline Congo red. Sections with the latter stain were studied in polarized light.

Electron Microscopy

For electron microscopic studies, small pieces of the adrenal cortex were fixed in 2% glutaraldehyde in 0.1M sodium cacodylate buffer, pH 7.2, containing 0.1M sucrose and postfixed for 90 minutes in 1% osmium tetroxide in the cacodylate buffer. The tissue pieces were then treated with 1% uranyl acetate in 50% ethanol overnight

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Table 1. *Frequency of Intracellular Amyloidlike Fibrils in Adrenal Cortex in Different Ages*

Age of patients (years)	Positive	Negative	% Positive
-60 (n = 11)	0	11	0
60–69 (n = 21)	0	21	0
70–79 (n = 35)	11	24	31
80–89 (n = 37)	21	16	57
90–99 (n = 10)	9	1	90
Total (n = 114)	41	73	36

and embedded in Agar 100 (Agar Aids, Stansted, Essex, England). Ultrathin sections were contrasted with lead citrate and studied in a JEOL 100 C electron microscope at 60 kV.

Immunohistochemistry

Immunohistochemical studies were performed on the adrenals of four patients with varying amounts of cytoplasmic amyloid inclusions. For negative control an adrenal gland from a newborn child without any inclusions was used.

Antibodies to human transthyretin (TTR) and to retinol-binding protein (RBP) were obtained from DAKO (Copenhagen, Denmark). Rabbit antibodies to cellular RBP (cRBP) were a gift from Dr. C Busch, Uppsala, Sweden. Rabbit antisera to amyloid protein AA,[3] the TTR-related amyloid protein ASc_1,[4] islet amyloid polypeptide (IAPP),[5] and to the subunit protein of isolated atrial amyloid (IAA)[6] have been characterized previously. As positive control for anti-TTR we used choroid plexus and for anti-RBP and anti-cRBP liver tissue was used. For anti-IAPP and anti-IAA subunit protein we used pancreatic tissue from a diabetic patient and atrial material from a patient with known local amyloidosis in the atria, respectively. For negative controls, the primary antiserum was replaced by nonimmunoserum.

Deparaffinized tissue sections were incubated with the primary antisera overnight at room temperature and subjected to the peroxidase-antiperoxidase method.[7] For visualization of the immune reaction, 3′,3′-diaminobenzidine (Sigma Chemical Co, St. Louis, MO) was used. All primary antisera were applied in dilution 1:200 to 1:800.

Results

Cytoplasmic inclusions with affinity for Congo red and a bright green birefringence after such staining often oc-

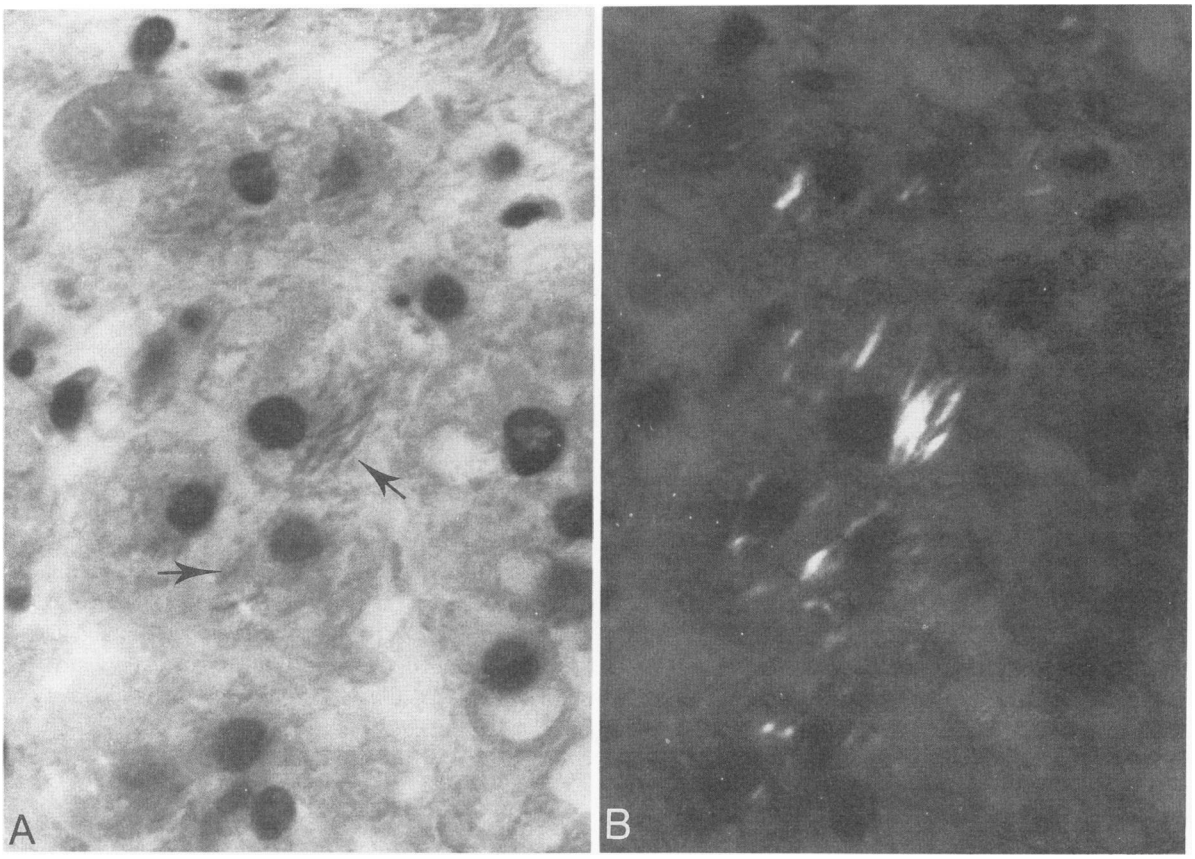

Figure 1. *Cortical epithelial cells some of which contain many needle-shaped amyloid inclusions (arrows) showing affinity for Congo red* **(A)** *and a bright green birefringence in polarized light after such staining* **(B).** *× 1200.*

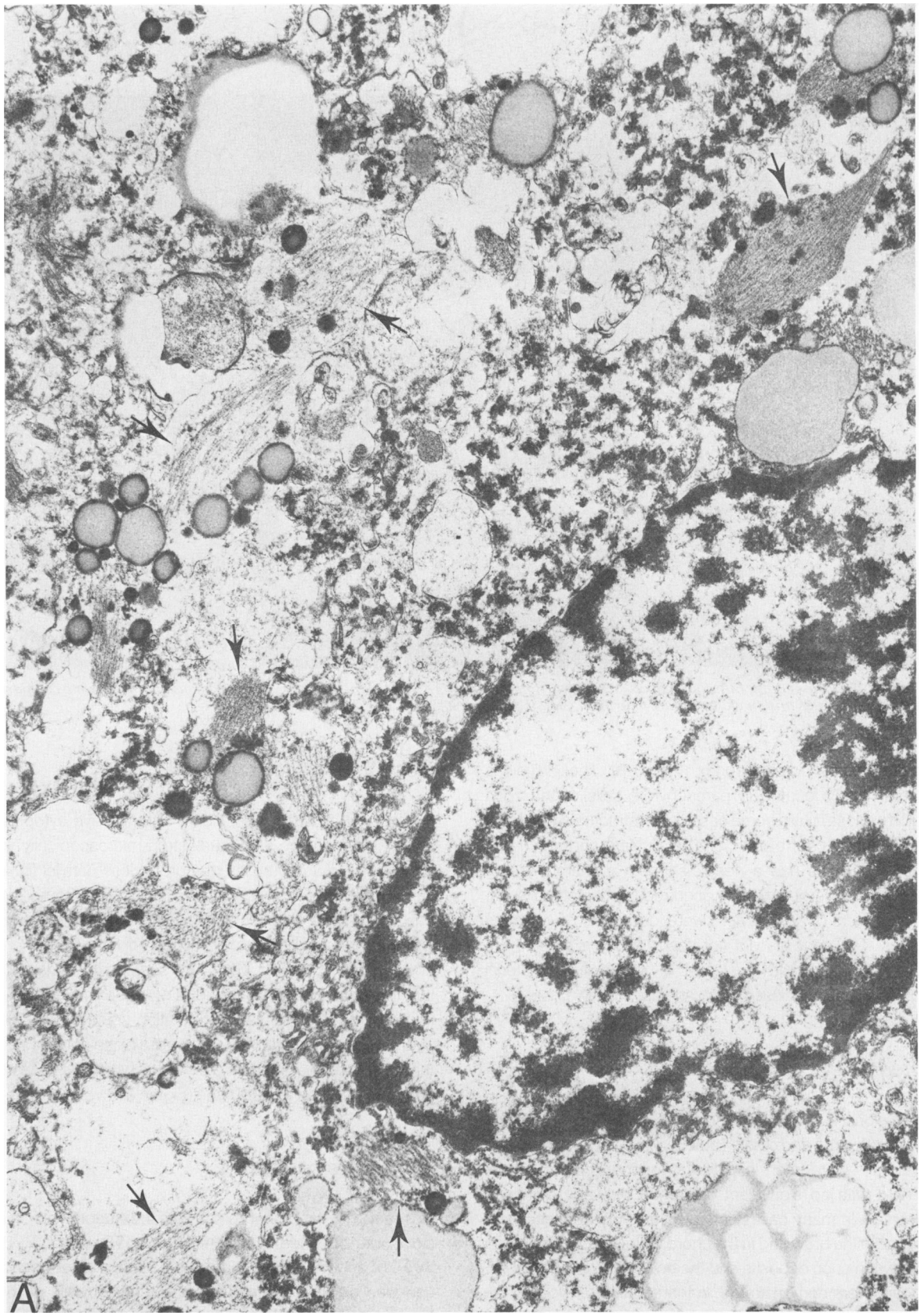

Figure 2. A: *Electron microscopic picture of an adrenal cell containing many amyloid inclusions (arrows). Most of the inclusions are in contact with lipid droplets and some are partially limited by a membrane. The fibrils are densely packed in some of the inclusions.* × *20,000.*

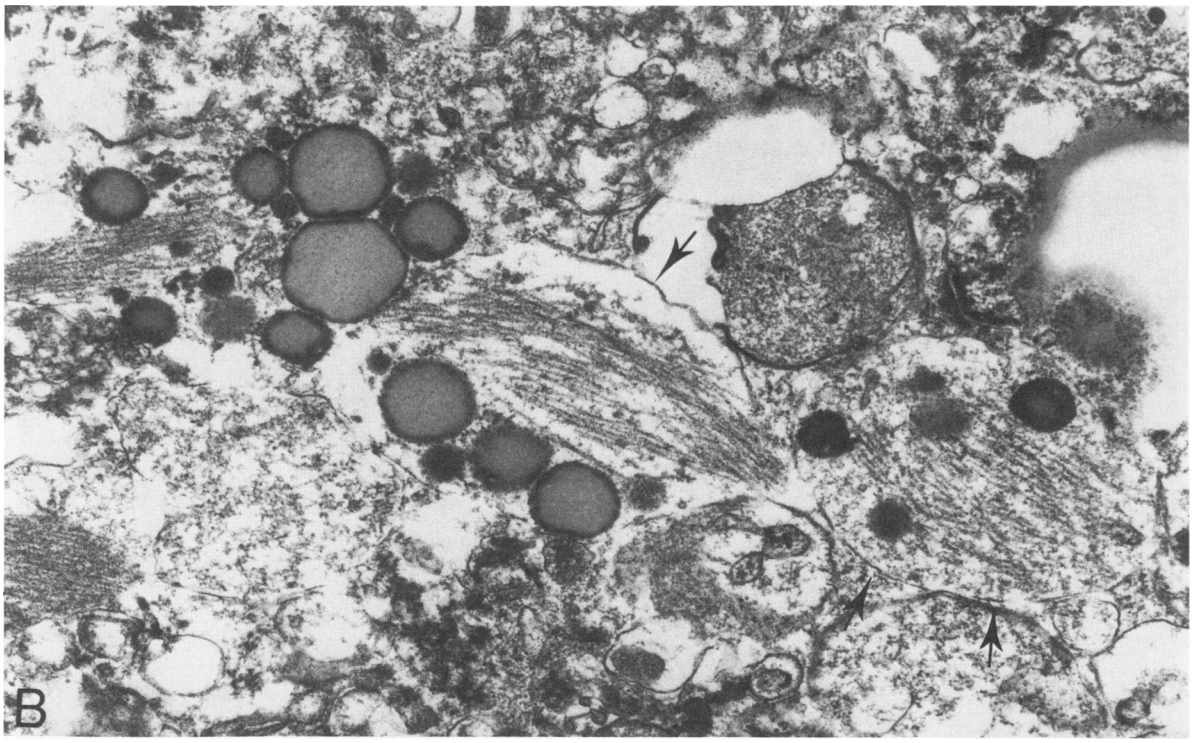

Figure 2. B: *Intracellular bundles of fibrils, at least partially limited by a membrane (arrows). Lipid droplets are in the same compartment as the fibrils and in close contact with them. Detail of Figure 2A, × 33,500.*

curred in the adrenal cortex (Figure 1). The inclusions were mainly located in parenchymal cells of the zona glomerulosa, but in some cases such structures were also identified in cells of the zona fasciculata and reticularis. The inclusions had a needle-shaped appearance and, sometimes, large amounts occurred in effected cells. Often clusters of cortical cells were loaded with such inclusions. The deposits did not seem to occur outside the cells, although the existence of extracellular material could not be dismissed with certainty in every case.

Frequency Study

Inclusions were identified in 41 of the 114 patients who were 70 years and older (Table 1). No intracellular adrenal deposits were seen in patients younger than 70 years. There was no significant difference between men and women. In many cases, localized amyloid deposits were found in the brain and in the choroid plexus. No correlation between such deposits and the occurence of adrenal deposits were demonstrated. In three patients, two with senile systemic amyloidosis and one with primary (AL) systemic amyloidosis, amyloid depositions in adrenal vessel walls were demonstrated. Only the patient with primary systemic amyloidosis also had intracellular adrenal deposits.

Electron-microscopic Findings

Adrenal cortex from two patients with massive cytoplasmic inclusions were studied electron microscopically. In both cases, many bundles of more or less parallel fibrils were identified in many cortical epithelial cells (Figure 2a). The fibrils were sometimes densely packed but more loosely arranged bundles also occured. The fibrils were about 12 to 15 nm in width and seemed to consist of two fine filaments with a diameter of approximately 6 nm (Figure 3). No regular periodic twisting could be demonstrated. Many lipid droplets occurred in close contact with the fibril bundles (Figures 2b and 3), which often were partially limited by a membrane (Figure 2).

Immunohistochemistry

No reaction with adrenal cells or with the intracellular inclusions was obtained when antisera to TTR, ASc1, RBP, IAPP, or subunit protein of IAA was used. A strong reaction was obtained within almost all cortical cells of the zona glomerulosa when anti-cRBP was used. This reaction was seen regardless of age or the occurrence of cytoplasmic inclusions. The reaction product was somewhat granular and seemed to be unevenly distributed in the cells. The intracellular inclusions did not react.

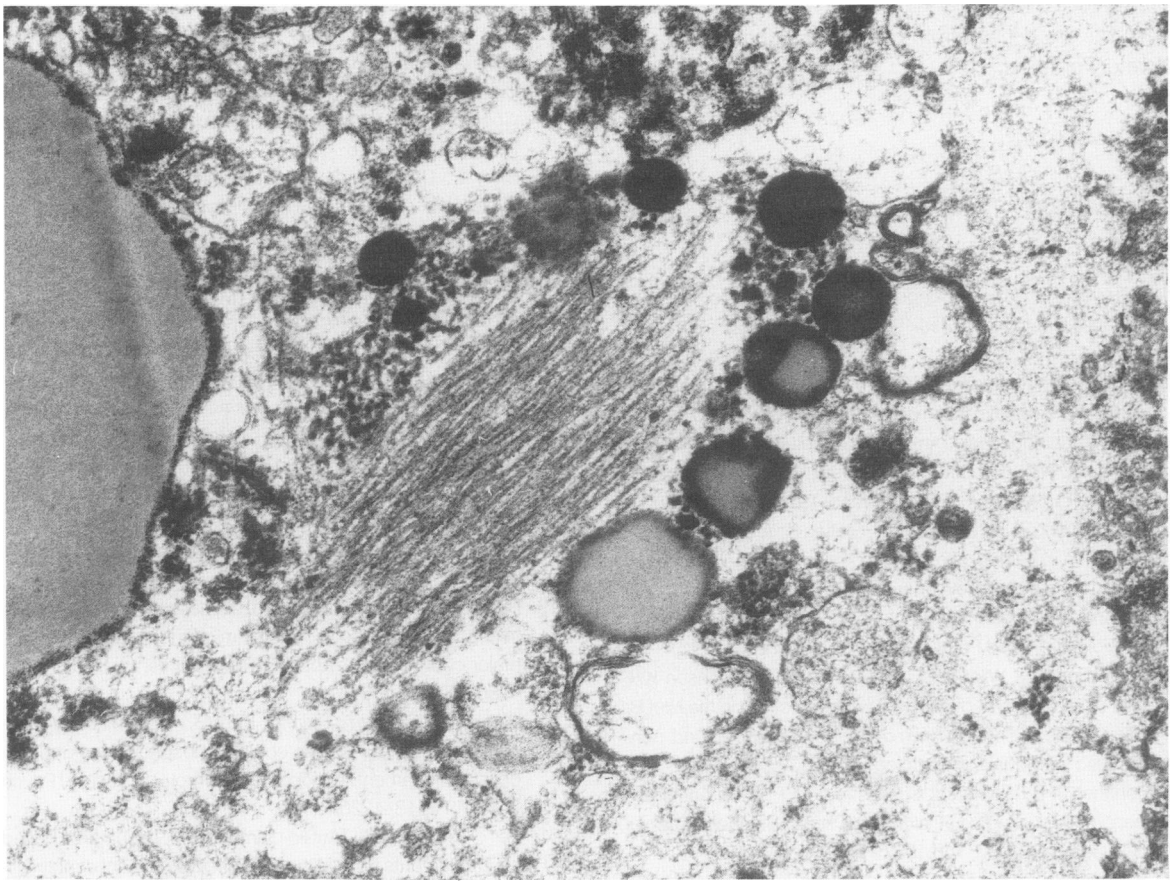

Figure 3. *A bundle of intracellular fibrils in contact with lipid droplets. × 81,000.*

Discussion

Small depositions of amyloid are very common in several tissues in elderly persons.[8] The nature of some of these have been elucidated during the last years. The deposits are mainly located extracellularly. Intracellular depositions with staining properties of amyloid occur in neurons in the cerebral cortex as neurofibrillary tangles,[9–12] in human upper cervical ganglia[13] and in the epithelial cells of the choroid plexus.[14–16] To these forms we now add the inclusions commonly found in the adrenal cortex.

The term *amyloid* is usually referred to as an extracellular material with certain staining properties, characteristic ultrastructural appearance, and typical X-ray diffraction pattern.[1] All amyloids that fulfill all of these criteria are, at least mainly, polymers of small proteins or polypeptides, usually with a molecular mass less than 20 kd.[1] Whether the intracellular fibrillar inclusions in the nervous tissue, the choroid plexus, and the adrenal cortex should be regarded as a form of amyloid can be a matter of discussion. They all exhibit green birefringence after staining with Congo red, have a fine fibrillar ultrastructure, and are resistant to solvents like other amyloid fibrils (Eriksson and Westermark, unpublished observation). Furthermore, in the case of neurofibrillary tangles, a β-pleated sheet conformation has been demonstrated.[17] It has not been shown in a definite way, however, that any of the intracellular inclusions are comprised of small proteins. Although the small β-protein, typical of the amyloid in congophilic angiopathy and in the plaque cores, has been reported to constitute the neurofibrillary tangles,[18] most evidence indicates that these inclusions, at least mainly, consist of the microtubule-associated protein tau.[19–24]

Amyloid deposits are known to occur in a variety of endocrine tissues. The fibril proteins that have been characterized from localized deposits in endocrine tissues have all originated from polypeptide hormones. Thus, in medullary carcinoma of the thyroid, the amyloid is derived from procalcitonin.[25] Amyloid in the islets of Langerhans and in insulin-producing tumors consists of the putative polypeptide hormone IAPP.[26] In isolated atrial amyloid (IAA), which is the most common amyloid form affecting the heart, the amyloid fibril is derived from atrial natriuretic factor (ANF).[6] The nature of the inclusions in the adrenal cortical cells is unknown and no reaction was obtained with the amyloid protein antisera used in this study. Re-

garding their intracellular nature, it is possible that these inclusions are derived from cytoskeletal rather than from secretory proteins.

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