AMYLOIDOSIS OF FUNCTIONING ISLET CELL ADENOMAS OF THE PANCREAS

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Conventional and electron microscopic studies of functioning pancreatic islet cell adenomas have shown the beta cells of these neoplasms, including the granules and organelles, to be similar to the beta cells of normal islets of Langerhans.[1,2] A feature characteristic of islet cell adenomas, however, has been the existence of a considerable amount of hyaline material, most of it lying between the basement membrane of beta cells and blood vessels.[2,3] On the basis of its electron microscopic characteristics, Lacy has stated that this substance is probably a protein, but that it does not have the characteristics of either collagen or reticulin. It does not appear to possess any insulin activity.[3] The purpose of this investigation is to attempt characterization, at least in part, of the nature of the hyaline substance.

Because the hyalin in these adenomas has some of the morphologic features of protein, we wished to learn specifically if it had any of the characteristics of amyloid. Amyloid can be most readily identified by its staining reactions. It binds Congo red and stains metachromatically with the rosaniline group of dyes, of which methyl violet is one. In addition, Vassar and Culling have shown that amyloid can be demonstrated with thioflavine S, a fluorescent stain.[4] Amyloid also reacts in positive manner to the periodic acid-Schiff (PAS) stain.[5] In this study we utilized these histochemical methods as well as electron microscopy to investigate the hyaline substance in a group of examples of islet cell adenoma.

MATERIAL AND METHODS

A recent case of pancreatic islet cell adenoma removed at operation from a 47-year-old woman was examined by electron microscopy, histochemical techniques and insulin assay. Tissues from 5 other functioning islet cell adenomas similarly removed were re-examined and studied by histochemical techniques. The age and sex of the patients, their symptoms, and the duration of their illnesses are shown in Table I.

For electron microscopy, the tissue was fixed in osmium tetroxide, embedded in methacrylate, sectioned with a Porter-Blum microtome and examined with an RCA Model EMU-3F electron microscope. Some of these sections were stained with lead hydroxide in an attempt to demonstrate particulate material suggestive of glycogen.

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This adenoma was also fixed in 3:1 acetic acid-methyl alcohol, embedded in paraffin, and sections stained by the hematoxylin and eosin, Congo red, methyl violet, thioflavine S, PAS (with and without previous diastase digestion) and Gomori's aldehyde fuchsin techniques. All staining methods were carried out as described by Lillie,[6] with the exception of the thioflavine S procedure; for this the method recommended by Vassar and Culling[4] was used. As controls for the amyloid stains, sections of liver and kidney from a case of primary amyloidosis were used.

TABLE I

CLINICAL DATA OF PATIENTS WITH FUNCTIONING PANCREATIC ISLET CELL ADENOMAS

Case no.	Age	Sex	Chief symptoms	Duration of symptoms
1	32	F	Stupor; confused state	2 mo.
2	36	M	Transient hemiplegia; clonic convulsions	4 mo.
3	47	F	Loss of consciousness; noisy behavior	10 mo.
4	47	F	Rigidity; choreiform movements	5 mo.
5	48	F	Weakness; diaphoresis	7 mo.
6	50	M	Confused state; diaphoresis	15 yr.

The other 5 adenomas examined were fixed in 10 per cent formalin; paraffin sections were stained with hematoxylin and eosin, Congo red, methyl violet and thioflavine S.

Insulin assay of the adenoma examined by electron microscopy was done by the method of Yalow and Berson.[7]

RESULTS

Electron Microscopy

Gray, moderately electron dense material lay between the basement membrane of the beta cells and the adjacent blood vessels (Fig. 1). In some sections this substance appeared fibrillar, but in most areas its consistency is better described as foamy. The characteristics of the beta cells themselves (Fig. 2) were similar to those of normal beta cells, except that the granules in most instances did not appear to be surrounded by sacs. Occasionally there were intracytoplasmic droplets of fat or some other equally dense osmiophilic material. Lead hydroxide staining failed to show any particulate substance indicative of glycogen within the beta cell cytoplasm. Although few beta granules were demonstrable by either electron or light microscopy, assay showed more insulin than it was possible to quantitate by the method used.

Conventional Microscopy

Hematoxylin and eosin stains of the recently removed adenoma showed a considerable amount of laminar hyaline material between vessel walls and the neoplastic beta cells (Fig. 3). Occasionally the substance appeared to be incorporated into the cytoplasm of tumor cells or macrophages. In all areas the deposit gave amyloid staining reactions with

Congo red (Fig. 4), methyl violet and thioflavine S. It stained moderately well with the PAS method both before and after diastase treatment. In addition, PAS stains showed a small content of granular material in beta cells. The Gomori aldehyde fuchsin stain showed the neoplastic beta cells to have few beta granules. No alpha granules were seen.

In the 5 other adenomas the hyalin was strongly positive to the Congo red stain in 2 cases, moderately positive in 2, and negative in only 1. In 2 of these 5 cases the deposit was definitely metachromatic with methyl violet and equivocally so in 2 others. In the 2 adenomas with definitely positive methyl violet metachromasia, the hyaline substance also stained in positive fashion with thioflavine S when examined by ultraviolet light. The staining reactions of all 6 cases are shown in Table II.

TABLE II

ISLET CELL ADENOMA. AMYLOID STAINS

Case no.	Congo red	Methyl violet	Thioflavine S
1	++*	++	++
2	+++	±	±
3†	+++	++	++
4	−	−	−
5	+++	±	±
6	++	−	−

* +++ = Strongly positive.
++ = Moderately positive.
± = Questionably positive.
− = Negative.
† Electron microscopy done in this case.

DISCUSSION

The results of this study suggest that at least in some functioning pancreatic islet cell adenomas the laminar hyaline material found between the basement membranes of beta cells and adjacent blood vessels had the characteristic qualities of amyloid. As seen by electron microscopy, the substance was similar to amyloid as observed in the renal glomerulus.[8] When examined by conventional microscopy, it stained with Congo red in 5 of 6 adenomas and gave positive reactions with both methyl violet and thioflavine S in 3 of 6 specimens.

The exact chemical nature of amyloid is not known, but it has been reported to be composed mostly of protein, with possibly a small amount of carbohydrate.[9] Because of the variable staining characteristics of different types of amyloid,[5] it is possible that its chemical composition is not constant. This could account for the variation in staining of the amyloid in the islet cell adenomas. The tinctorial qualities did not appear

to correlate with the duration of the tumor or the severity of the patient's symptoms, although neither of these features were susceptible to accurate assessment. The amyloid in the islets of Langerhans in diabetes appears to have constant staining characteristics.[10] It would be of interest to know the relationship, if any, of this amyloid to that in islet cell adenomas.

Some studies have suggested that globulin constitutes part of the chemical composition of amyloid.[11,12] This has led to the suggestion that amyloid may be an antigen-antibody precipitate; this theory is by no means proven.[13] Using fluorescent antibody technique, Lacy has obtained data suggesting that the insulin of islet cell adenomas may be immunologically different from normal human insulin.[2,3] It is tempting to consider the possibility that the amyloid found in the adenomas in this study might represent an antigen-antibody precipitate in some way related to an immunologically abnormal insulin released by neoplastic beta cells. Confirmation of this hypothesis would obviously require further investigation.

In the present study and in that of others, the ultrastructure of beta cells in normal islets of Langerhans and in functioning beta cell adenomas appears to be the same. Thus, the uncontrolled release of insulin from islet cell adenomas does not appear to be explained by any morphologic peculiarities in the neoplastic beta cells. It is difficult to consider the amyloid to be responsible for an uncontrolled insulin release. Assay of the fibrillar (presumably amyloid) substance in adenomas has demonstrated no insulin activity; thus it apparently does not serve as a storage area for insulin released from the beta cells.

Summary

The structure of beta cells in functioning pancreatic islet cell adenomas is similar to that of normal beta cells. An unusual feature of the adenomas, however, is the presence of a hyaline substance lying between beta cells and adjacent blood vessels. Electron microscopic examination of a functioning islet cell adenoma showed this deposit to have, in general, the ultrastructure of amyloid. Congo red, methyl violet and thioflavine S stains showed that in 3 of 6 functioning islet cell tumors (including the one examined by electron microscopy) the hyaline substance had the tinctorial reactions of amyloid; in only 1 of the 6 were all 3 amyloid stains negative.

The exact nature of amyloid is not known; it is possible, however, that it may represent an antigen-antibody precipitate. If this is so, the amyloid found in islet cell adenomas may possibly be related to an immunologically abnormal insulin.

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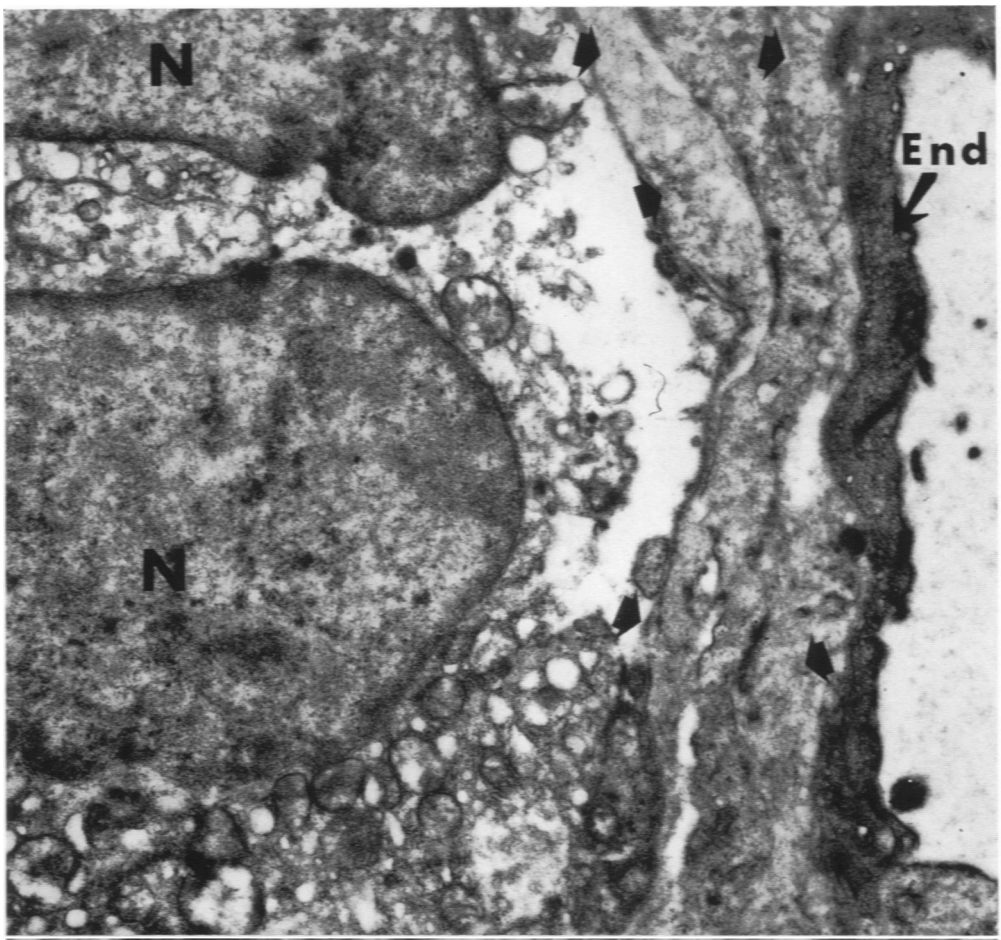
[*Illustrations follow*]

Legends for Figures

Fig. 1. A hyaline substance (arrows) is interposed between the basement membrane of a capillary and the membranes of beta cells. Empty spaces in the cytoplasm of these cells probably are artifacts. N = nucleus; End = endothelium. × 16,000.

Fig. 2. A portion of the cytoplasm of a neoplastic beta cell. The endoplasmic reticulum (E. ret.) is vesiculated but still contains numerous ribonucleic acid granules of Palade attached to its membranes. Mitochondria (Mt) are slightly altered and show patchy, clear areas in their matrix. Beta granules (Gr) in the cytoplasm are not surrounded by detectable sacs. × 32,000.



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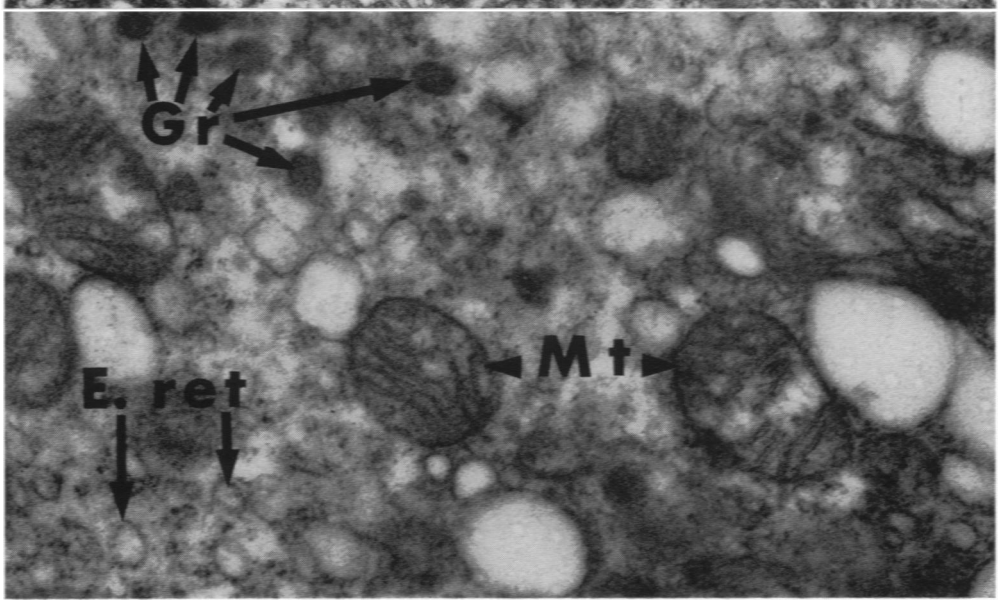

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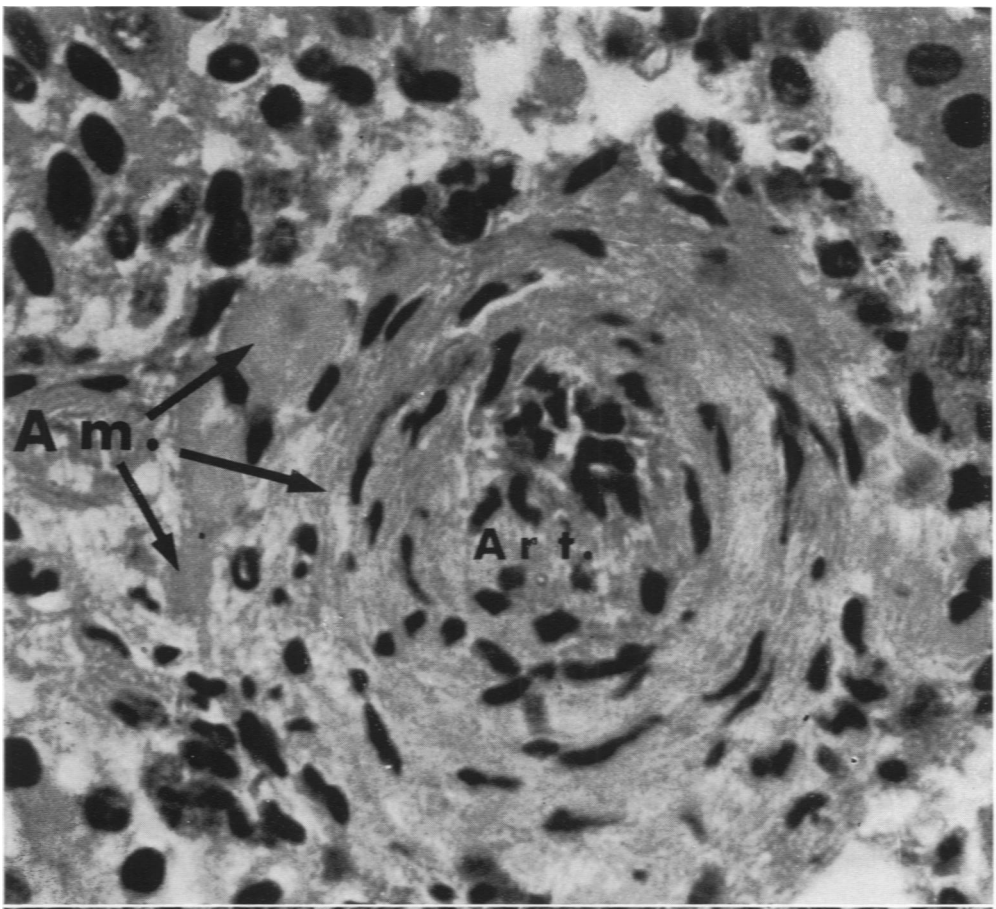
FIG. 3. A portion of an islet cell adenoma. The hyaline deposit (Am) in the wall and around an almost completely obliterated arteriole (Art) stained with Congo red and was metachromatic with methyl violet. It also displayed a characteristic fluorescence after staining with thioflavine S. Hematoxylin and eosin stain. × 2,000.

FIG. 4. The amyloid (Am) deposited between the beta cells and an adjacent blood vessel exhibits Congo red staining. × 600.



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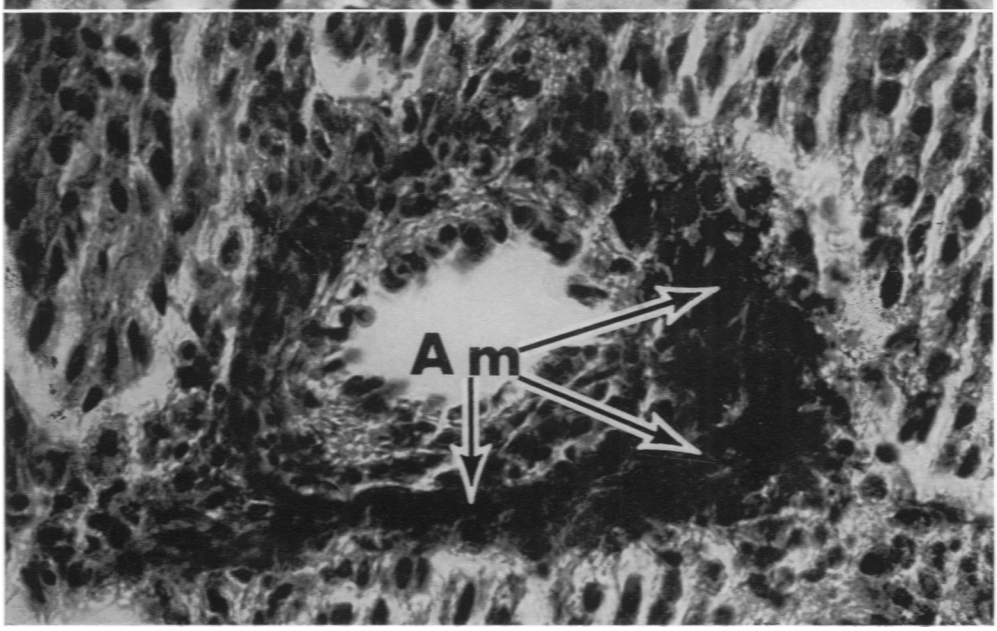

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