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Fine Structure of Islets of Langerhans in Insular Amyloidosis*

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Summary. The islets of Langerhans of diabetic and non-diabetic patients with different degrees of islet amyloidosis were studied by electron microscopy. The islet amyloid exhibited the typical fine fibrillar ultrastructure and was mainly located interstitially. Adjacent to the β cells the amyloid fibrils were often highly orientated perpendicularly to the cell surface and bundles of amyloid fibrils entered in deep plasmalemmal invaginations of the cells. This was more rarely seen in other types of cell. The epithelial cells exhibited no signs of increased activity. Macrophages were common in the amyloid masses. Amyloid occurred in invaginations of these cells but usually the fibrils showed no orientation. The capillaries, the fibrocytes and the mast cells were not so closely related to the amyloid. These findings probably indicate that the amyloid of the islets of Langerhans is a product of degenerating β cells even if other possibilities are not excluded.

Zusammenfassung. Die Ultrastruktur der Langerhansschen Inseln bei der Inselamyloidose. Die Langerhansschen Inseln diabetischer und nicht-diabetischer Patienten mit verschiedenen Schweregraden einer Inselamyloidose wurden elektronenmikroskopisch untersucht. Das Inselamyloid zeigte eine typische feinfibrilläre Ultrastruktur und war vorwiegend interstitiell lokalisiert. In der Nachbarschaft der β-Zellen waren die Amyloidfibrillen oft senkrecht zur Zelloberfläche orientiert, wobei Bündel von Amyloidfibrillen in tiefe Invaginationen der Zellgrenzmembran hineinragten. Diese Beobachtung fand sich selten bei den anderen Inselzelltypen. Die Epithelzellen zeigten keine Hinweise auf eine gesteigerte Aktivität. Im Bereich der Amyloidmassen fanden sich in der Regel Makrophagen. Das Amyloid war in den Invaginationen dieser Zellen erkennbar, allerdings gewöhnlich nicht in paralleler Orientierung. Capillaren, Fibrocyten und Mastzellen waren dem Amyloid weniger dicht benachbart. Aus den Befunden wird der Schluß gezogen, daß das Amyloid der Langerhansschen Inseln ein Produkt degenerierender β-Zellen darstellt. Andere Möglichkeiten der Entstehung lassen sich allerdings nicht ausschließen.

The resemblance between hyalin in human islets of Langerhans and amyloid was pointed out at an early date (Mallory, 1925; Gellerstedt, 1938; van Beek, 1939; Arey, 1943; Ahronheim, 1943). When it was found that islet hyalin displayed the same staining reaction with Congo red as amyloid (Ehrlich and Ratner, 1961) and the fine fibrillar ultrastructure of hyalin was shown (Lacy, 1964), the amyloid nature of hyalin was fairly generally accepted.

There is increasing evidence for *in situ* production of amyloid (Teilum, 1964; Ranløv and Wanstrup, 1967, 1968), and the close topographic relationship between amyloid deposits and cells of the reticuloendothelial system is suggestive of

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formation of amyloid by these cells (Gueft and Ghidoni, 1963; Sorenson *et al.*, 1964; Cohen, 1965; Cohen *et al.*, 1965; Ranløv and Wanstrup, 1967, 1968), at least in systemic amyloidosis.

The significance of the deposition of amyloid in the islets of Langerhans is unknown. It is unlikely that it is any major cause of diabetes (Lacy, 1970). The islet amyloid is, however, in some way related mainly to maturity onset diabetes. Only few and fairly cursory investigations have been made concerning the ultrastructure of the islets in islet amyloidosis (Kawanishi *et al.*, 1966; Yamada, 1968; Kohama *et al.*, 1969). The aim of this investigation was to study the relationship between the different islet cells and the amyloid and to describe this in detail.

Material and Methods

This study was carried out on an autopsy material of patients, the data for whom are given in Tables 1 and 2. Ten patients had clinically manifest maturity onset diabetes mellitus. In the twelve non-diabetic patients manifest diabetes had been excluded by several glucose-free urine specimens and often at least one normal blood glucose determination result.

Table 1. Diabetic persons

Case	Sex	Age at death (years)	Duration of diabetes (years)	Islet amyloid in % of islet area (approx.)	% involved islets
1	♂	69	6	36	97
2	♀	76	13	0.5	8
3	♀	66	11	1	20
4	♂	77	4	1	13
5	♀	68	4	0	0
6	♀	76	11	36	97
7	♀	78	5	28	91
8	♀	74	26	42	100
9	♂	82	16	8	65
10	♂	68	10	40	99

Table 2. Non-diabetic persons

Case	Sex	Age at death (years)	Islet amyloid in % of islet area (approx.)	% involved islets
1	♂	89	0.5	2
2	♂	84	0	0
3	♂	67	0.5	1
4	♀	83	1	21
5	♀	78	0	0
6	♀	86	0	0
7	♂	71	36	97
8	♂	77	1	23
9	♂	60	0.5	6
10	♀	67	1	19
11	♂	69	0.5	12
12	♀	80	0	0

The time between death and autopsy varied between 3 and 9 hours. Pending autopsy except for the two first hours, the bodies were kept at +4° C. The pancreas was removed immediately at autopsy. Small pieces from the pancreatic tail were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4 for 4 hours. After rinsing in 0.1 M sucrose the specimens were post-fixed in 1% osmium tetroxide for 90 minutes. All steps in the fixation process took place at +4° C. The specimens were dehydrated in graded concentrations of ethanol and embedded in Epon (Luft, 1961). About 1 μ thick sections were studied in a phase contrast microscope or, after Congo red staining (Shirahama and Cohen, 1966), in a polarization microscope. Thin sections of relevant areas were cut on a LKB Ultrotome, stained with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963) and examined in a Zeiss EM 9 electron microscope at 60 kV.

The degree of amyloidosis was determined as described previously (Westermark, 1972a) on Bouin- and formalin-fixed Congo red stained sections. As is seen from the tables the degree of amyloidosis was generally much lower in the non-diabetic than in the diabetic patients.

Observations

Changes considered to be due to post mortem autolysis were always found, but in varying degrees. The endoplasmic reticulum was moderately dilated but the ribosomes of the rough endoplasmic reticulum were usually intact. The mitochondria showed diminished electron density and appeared swollen but with at least partially retained cristae. Large vacuoles sometimes appeared in the cytoplasm but this finding was most common in the exocrine cells. The granules seemed intact both in endocrine and exocrine cells. The nuclei displayed aggregation of the chromatin. The cell membranes were sometimes indistinct especially in exocrine cells, but appeared intact in most regions. In areas without amyloid the basement membranes were always intact.

Deposits of amyloid were observed between the basement membranes of the endocrine cells and the capillaries. The amount of amyloid varied greatly in different parts even of the same islet. The amyloid had a fine fibrillar structure with about 100 Å thick, non-branching fibrils running in different directions (Fig. 1). There was no difference between diabetic and non-diabetic persons regarding the structure of the amyloid or the relation of amyloid to different types of cell.

Epithelial Cells

Islets without Amyloid

Three different epithelial islet cells were easily distinguished, as described previously (cf. Björkman *et al.*, 1966; Grimelius, 1969). The capillaries and the interstitial cells were readily identified and had an ultrastructure similar to that previously described (cf. Westermark, 1972b). There was no definite difference either between islets from diabetics and non-diabetics or between islets from patients with amyloidosis in other islets and islets from patients without any islet amyloidosis.

Islets with Small or Moderate Amyloid Deposits

The α_1 cells showed no special features. They were always heavily granulated as is normally seen. Only rarely were α_1 cells found close to amyloid deposits.

The α_2 cells close to amyloid were rich in granules of normal appearance. As in the β cells the endoplasmic reticulum was sparse. The Golgi zone was almost never seen. There were many lipoid bodies and the nuclei were often pyknotic.

β cells in the vicinity of amyloid deposits were usually heavily granulated. The overwhelming majority of the granules had a dark irregular core and a wide sac. Granules with faint granular contents were sparse, as well as the endoplasmic

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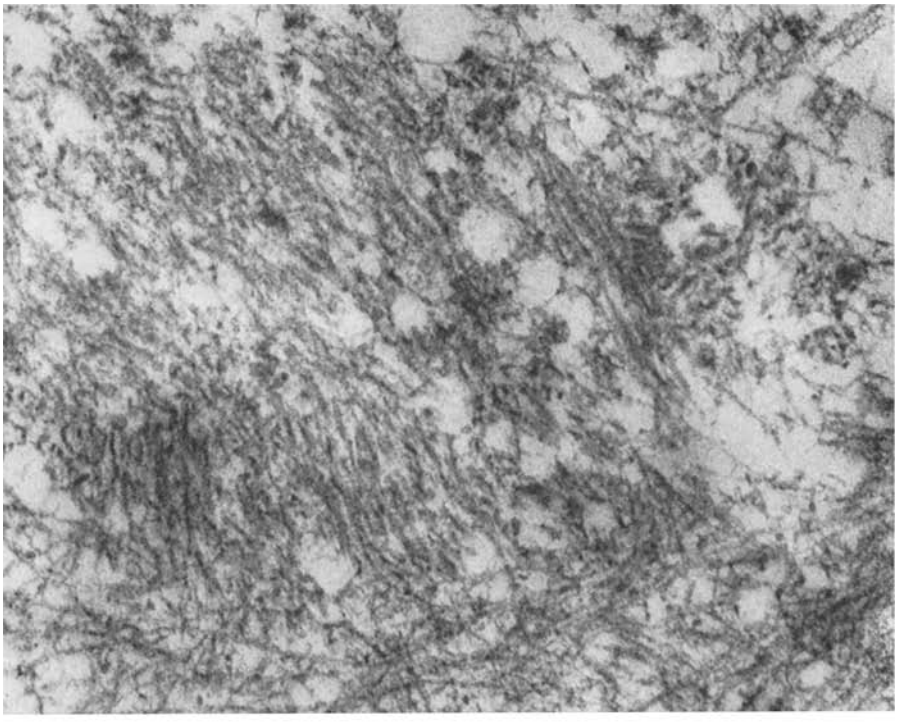

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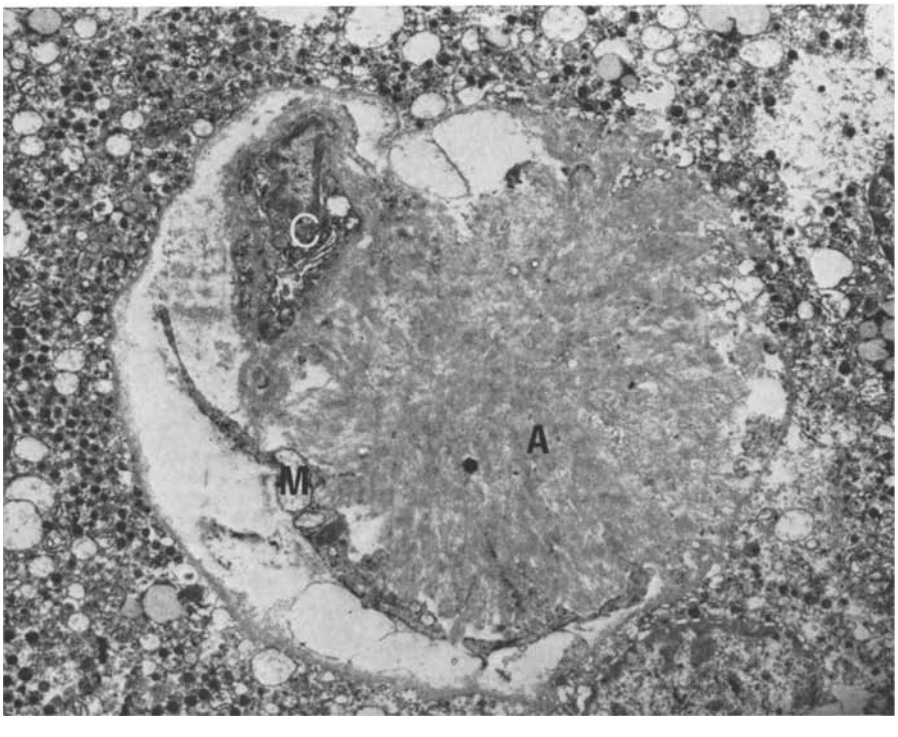

Figs. 1 and 2

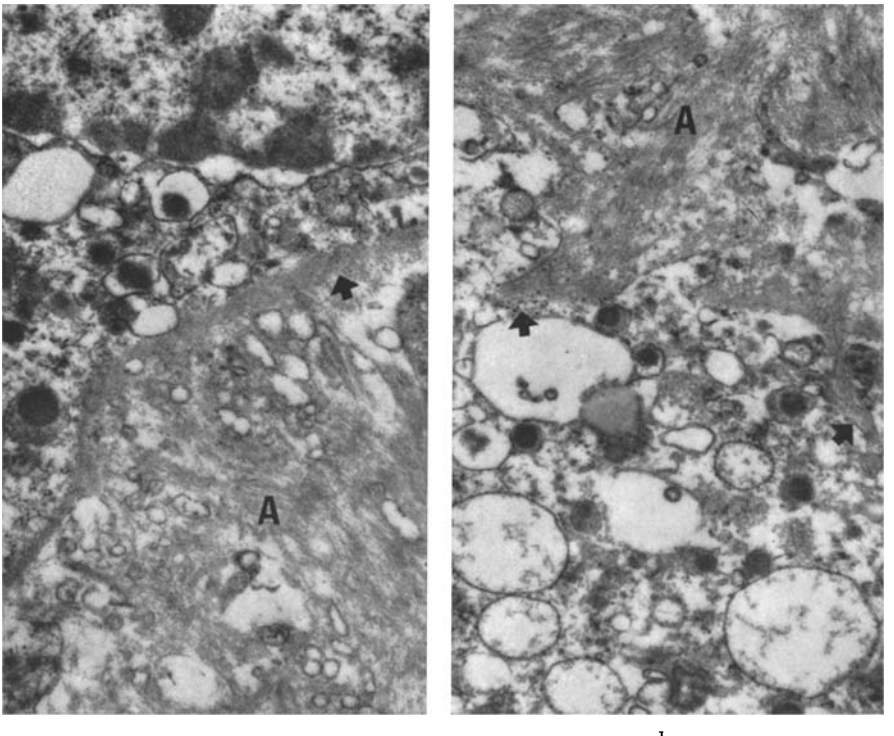

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Fig. 3. a β cell clearly demarcated from amyloid (A) by an intact basement membrane (arrow). The amyloid fibrils are usually orientated perpendicular to the cell surface. Numerous membrane limited vesicles without obvious contents are present in the amyloid. Moderate amyloidosis. Diabetic patient, 7 hours after death. ×19500. b β cell close to amyloid (A). The basement membrane is lost in some areas and bundles of parallel amyloid fibrils lying perpendicular to the cell surface run into membrane limited invaginations (arrows) in the cytoplasm. Moderate amyloidosis. Diabetic patient, 7 hours after death. ×16000

reticulum. The Golgi zone was rarely seen. The mitochondria showed no special features. Lipoid bodies occurred in large quantities. The nuclei were pyknotic.

The amyloid was almost exclusively interstitial, lying between the two basement membranes (Fig. 2). In many areas the amyloid close to β cells and sometimes to α_2 cells contained large quantities of small vesicles limited by a membrane and without obvious contents (Figs. 3a, b). Such vesicles also occurred but to a lesser degree at some distance from the cells (Fig. 3a). Islet cells adjoining areas with vesicles often had an empty appearance with very few

Fig. 1. Amyloid with non-branching, randomly distributed fibrils about 100 Å thick. Non-diabetic patient, 6 hours after death. × 69500

Fig. 2. Interstitially located amyloid (A) with a capillary (C) and a macrophage (M) on one side and epithelial cells on the other. The basement membrane of the epithelial cells is distinct in most areas. Small vesicles are numerous within the amyloid. Moderate amyloidosis. Diabetic patient, 7 hours after death. ×5000

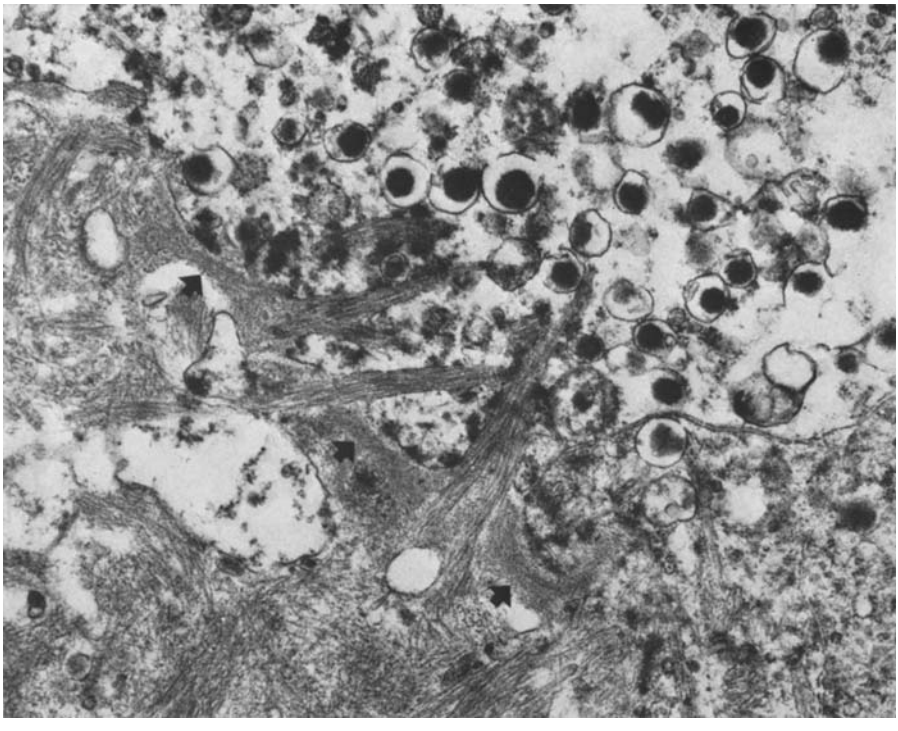

Fig. 4. β cell close to amyloid deposits. Bundles of parallel amyloid fibrils are seen in membrane limited invaginations in the cell. Between the invaginations the basement membrane is intact (arrows). Slight amyloidosis. Non-diabetic patient, 6 hours after death. $\times$ 19500

remaining organelles, including granules (Fig. 2). The nuclei were pyknotic but not more so, however, than other islet cells near the amyloid. The basement membrane of the epithelial cells was usually intact even when it was in close contact with the amyloid. Sometimes, however, it was indistinguishable or occasionally split up into two parts. Between these parts small vesicles, granules and isolated mitochondria were seen. Even in such parts the cell membrane was often intact.

The amyloid close to β cells was often highly orientated with bundles of parallel fibrils running perpendicular to the cell membrane (Figs. 3a, b, 4). Small bundles of highly orientated amyloid fibrils often seemed to penetrate the basement membrane and projected into deep plasmalemmal invaginations of the β cells (Figs. 3b, 4). The basement membrane between the amyloid bundles was preserved. There was no relation between the amyloid and any particular part of the cells. Occasionally amyloid bundles entered α_2 cells and very rarely also α_1 cells.

In islets containing amyloid, cells at some distance from the deposits showed no alterations.

Islets with Massive Amyloidosis

Most of the β cells showed alterations. The border between the amyloid and the β cells was usually blurred and in such areas the cell membranes and the

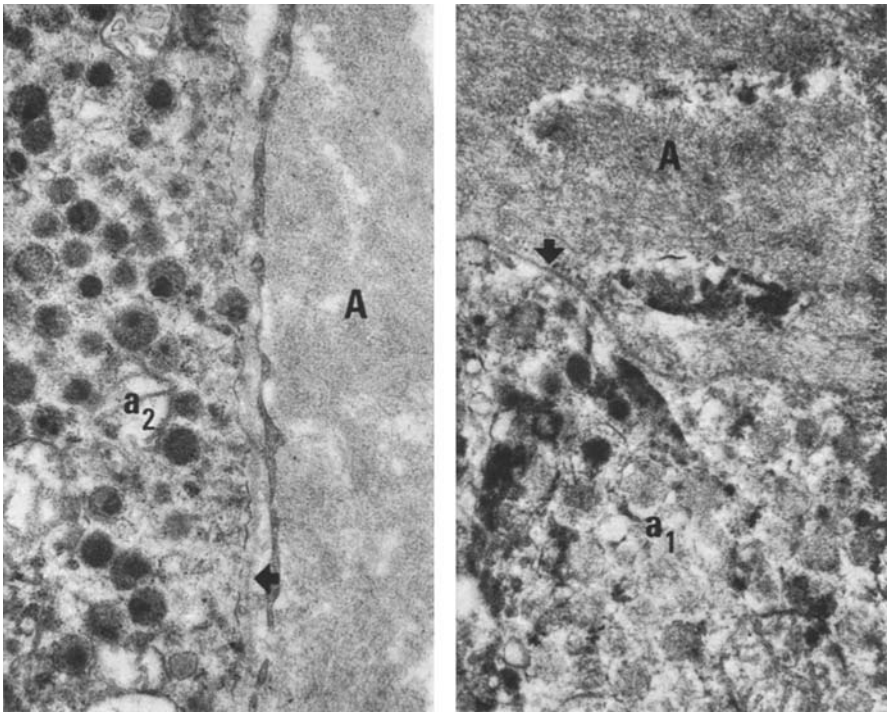

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Fig. 5a and b. Amyloid (A) close to α_2 (a_2) and α_1 (a_1) cell, respectively. The fibrils are orientated at random and do not penetrate the basement membranes (arrows) which are intact. Massive amyloidosis. Diabetic patients, 7 and 4 hours after death, respectively. ×20500 and 19000

basement membrane had disappeared. Groups of amyloid fibrils were highly orientated when they were adjacent to the β cells. Many bundles of amyloid fibrils were orientated perpendicular to the cell surface and penetrated deeply into the cells. These bundles were sometimes limited by a membrane. The cell organelles showed no special features when compared with cells in islets with slight amyloidosis. The nuclei were highly pyknotic.

The border between the α_2 cells and the amyloid was usually sharp, with the cell membranes and basement membrane preserved (Fig. 5a). The amyloid fibrils did not so often display the orientation seen in the vicinity of β cells, and often lay parallel to the cell membrane. Sometimes, however, the cell and basement membranes were broken through and bundles of amyloid perpendicular to the cell surface seemed to enter the cytoplasm as in β cells. The α_2 cells were rich in granules. The nuclei were pyknotic. The cell organelles showed no special features.

The α_1 cells were more rarely seen in contact with amyloid. The cells usually seemed to be unaltered, with the cell and basement membranes intact (Fig. 5b), but on rare occasions bundles of amyloid fibrils seemed to enter the cytoplasm of the α_1 cells.

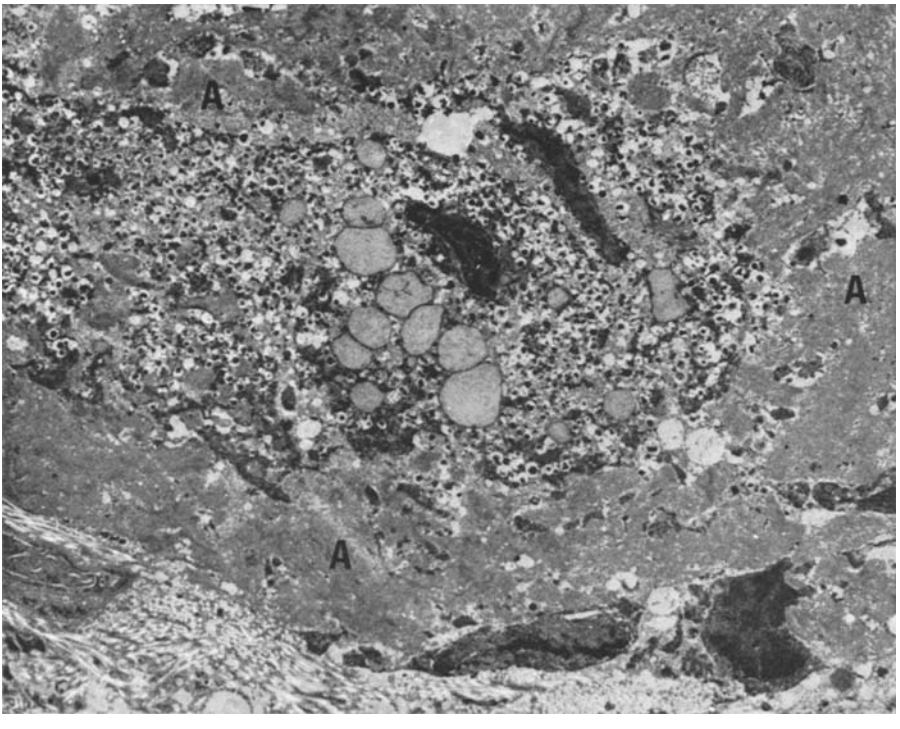

Fig. 6. Islet with highly degenerated β cells which are disintegrating. Remnants of cells are numerous in the amyloid (*A*) close to the islet cells. Massive amyloidosis. Diabetic patient, 9 hours after death. $\times 4500$

Some islets were occupied by very large amyloid deposits. In these islets intact β cells were rarely found. Most of the β cells showed signs of severe degeneration with many fragments lying in the amyloid masses (Fig. 6). These fragments often contained large quantities of granules of normal appearance. α_1 cells and α_2 cells often showed the same disturbances but to a lesser degree.

Capillaries

In islets with small or moderate amounts of amyloid the deposits were often situated between the islet epithelial cells and the capillaries (Fig. 2). The amyloid fibrils exhibited no definite polarity towards the capillary basement membranes (Fig. 7a), which were always distinct also around the pericytes. The endothelial cells and the pericytes showed no abnormal features.

In islets with heavier amyloid deposits the capillaries were often more or less surrounded by amyloid (Fig. 8). However, the greatest amount of amyloid occurred between the capillaries and the epithelial islet cells. Also in these heavily amyloid-infiltrated islets the capillary basement membrane appeared to be intact except for some small areas especially on the interstitial side of the pericyte, where

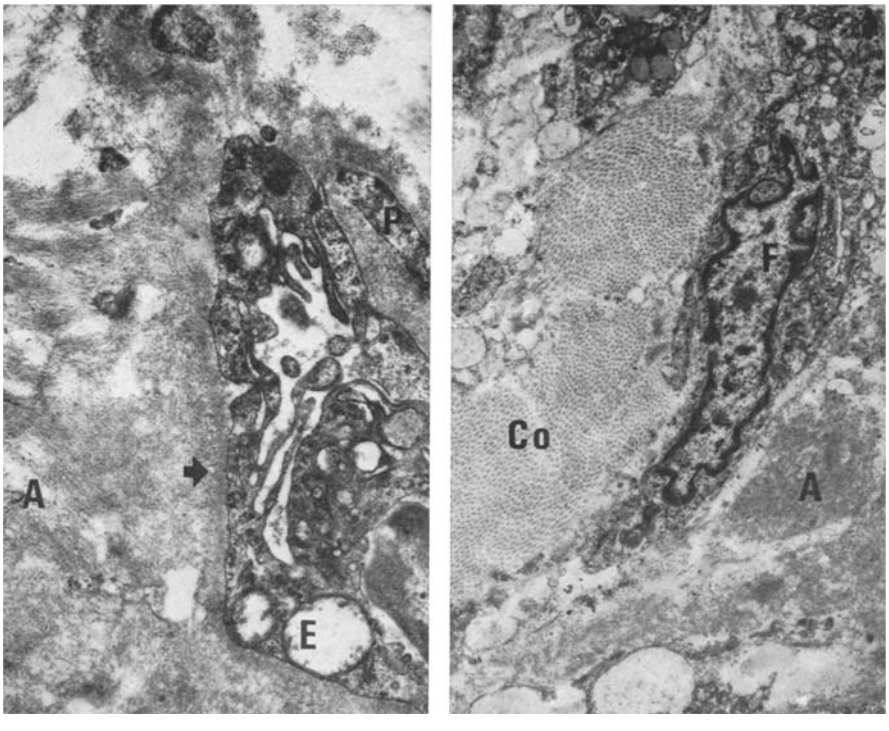

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Fig. 7. a Capillary adjacent to amyloid (*A*). A pericyte (*P*), endothelial cells (*E*) and basement membrane (arrow) seem intact. Moderate amyloidosis. Diabetic patient, 7 hours after death. × 18500. b Fibrocyte (*F*) adjacent to collagen (*Co*) and amyloid (*A*). The cell has an inactive appearance with a sparse cytoplasm and few organelles. Slight amyloidosis. Non-diabetic patient, 4 hours after death. × 6000

sometimes it was indistinguishable. The endothelial cells and the pericytes were often poor in organelles and frequently contained residual bodies. Fenestrae were seen as normally.

Fibrocytes

Fibrocytes were seen in a moderate number in all islets. Most of them were long and narrow with a spindle shaped nucleus and thin slender cytoplasmic processes (Fig. 7 b). In the cytoplasm, the endoplasmic reticulum, the mitochondria, the Golgi complex and the free ribosomes showed no noteworthy changes. Microfilaments were numerous. Fibrocytes with a more active appearance were sometimes found in areas with fibrosis.

Fibrocytes often occurred in contact with amyloid deposits (Fig. 7 b). Usually the cell borders were sharp and without orientation of the amyloid fibrils in the vicinity.

In heavy deposits of amyloid scattered fibrocytes were found. These fibrocytes had an inactive appearance and had many attenuated cytoplasmic processes.

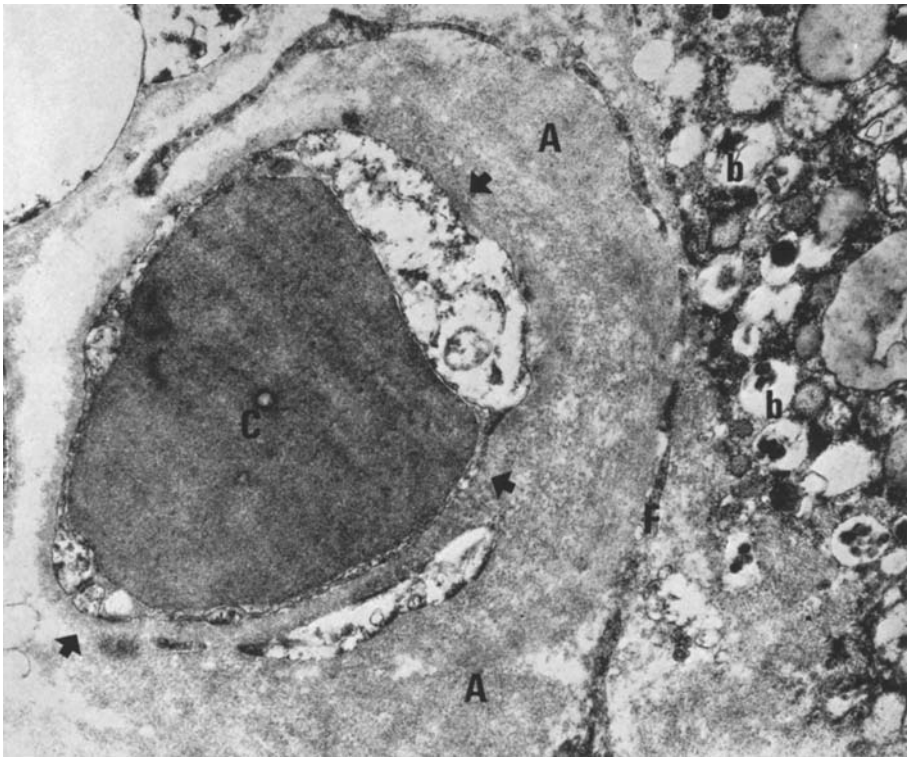

Fig. 8. Capillary (*C*) almost surrounded by amyloid (*A*). The endothelial cells are separated from the amyloid by an intact basement membrane (arrows). $b = \beta$ cell. Massive amyloidosis. Diabetic patient, 9 hours after death. ×16000

Macrophages

Cells which could be classified as macrophages (De Petris, 1962; Dumont, 1969) were found in a fairly large quantity in islets with amyloid deposits. In islets with small amyloid deposits many of the macrophages had an active appearance (Fig. 9a). The cytoplasm was abundant and the cytoplasmic organelles were well developed. Thus the rough-surfaced endoplasmic reticulum was conspicuous and sometimes showed prominent cisternae. The Golgi complex was large and the free ribosomes were numerous. Lysosome-like structures and residual bodies occurred in a moderate number. The mitochondria were often rather large. The cells were round or irregular in shape.

Macrophages in close contact with amyloid usually had a less active appearance (Fig. 9b) and often exhibited shallow or deep membrane-limited invaginations containing amyloid. The amyloid fibrils in these invaginations were sometimes orientated parallel to the longitudinal axis of the invaginations (Fig. 10a). However, the fibrils often showed no special orientation. Usually the cell membrane was recognizable where it was in contact with amyloid but there were small

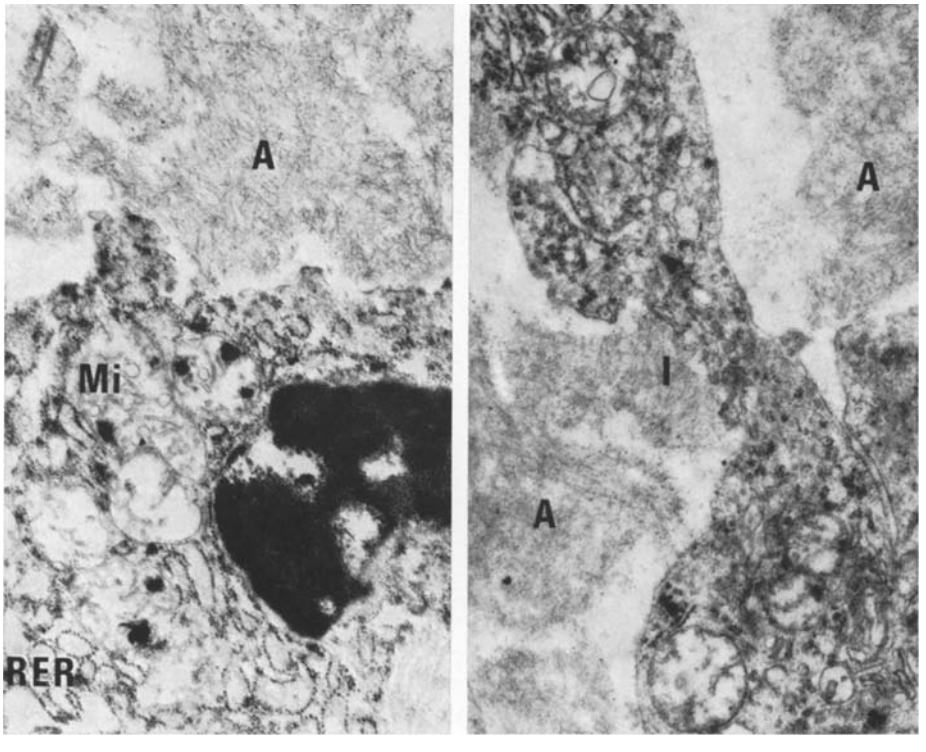

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Fig. 9. a Mesenchymal cell, probably a macrophage, close to amyloid (*A*). The cell has an active appearance with abundant cytoplasm in which the endoplasmic reticulum (*RER*) is well developed and the mitochondria (*Mi*) are large. Moderate amyloidosis. Diabetic patient. 7 hours after death. ×16000. b Macrophage in amyloid deposits (*A*). It has a moderately developed endoplasmic reticulum. A shallow amyloid-filled invagination (*I*) is seen. Moderate amyloidosis. Non-diabetic patient, 4 hours after death. ×15500

areas where the plasma membrane was indistinct. This was most often seen in the deepest part of the invaginations.

In large deposits some macrophages appeared to be merged in the amyloid (Fig. 10b). Many macrophages were particularly irregular in shape and sometimes long cell processes penetrated into the surrounding amyloid. The cells looked degenerated with an irregular nucleus and a reduced cytoplasm with a dense appearance, a sparse endoplasmic reticulum and an insignificant Golgi complex (Fig. 11).

Mast Cells

Most of the mast cells found in the vicinity of amyloid exhibited the typical features of intrainsular mast cells (Westermark, 1972b). Thus there were numerous mitochondria, a sparse endoplasmic reticulum and an only rarely identified Golgi complex. The granules were usually rather sparse. The granules were fairly small,

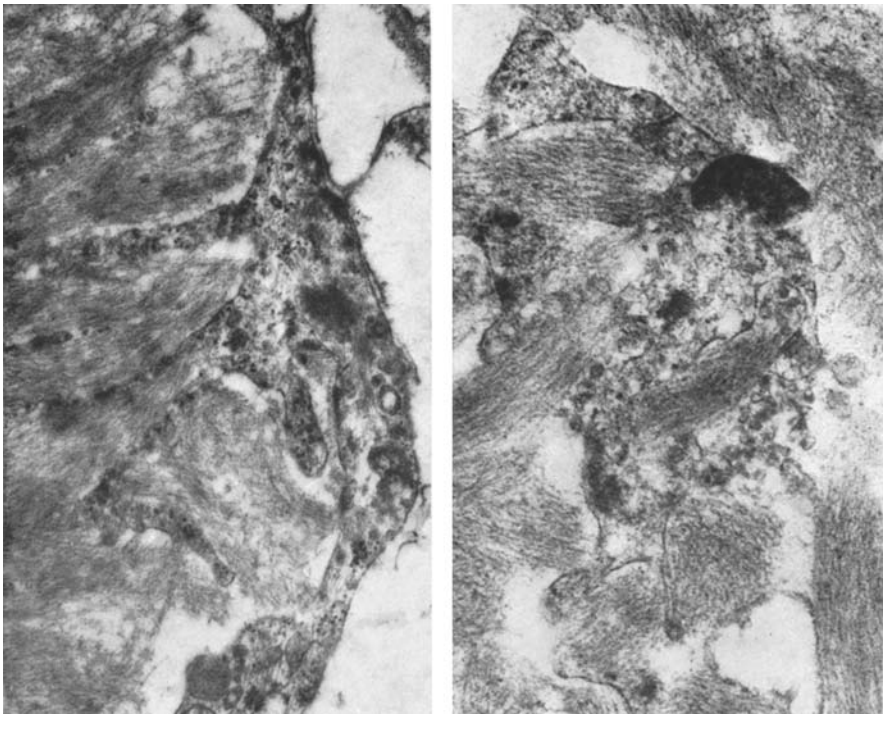

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Fig. 10. a Part of a macrophage with deep membrane limited invaginations in the cytoplasm or alternatively long slender cytoplasmic processes into the amyloid. In the cytoplasm microfilaments are numerous. Note the orientation of the amyloid fibrils towards the plasma membrane. Moderate amyloidosis. Diabetic patient, 7 hours after death. ×13500. b Part of a macrophage, merged in the amyloid. Membrane limited invaginations are distinct. Massive amyloidosis. Diabetic patient, 7 hours after death. ×15000

most of them $< 0.5\ \mu$ in diameter. Larger granules, with a diameter up to $0.9\ \mu$, were somewhat more commonly found than in islets without amyloid deposits. Most of the granules showed a pure lamellar structure. However, granular and crystalline areas were definitely more common than in mast cells of non-amyloidotic islets.

Mast cells in large amyloid deposits showed degenerative changes. Amyloid fibrils without any particular orientation seemed to penetrate the cell membrane over wide areas and the organelles including the granules had partially lost their structures (Figs. 12a, b).

Discussion

Electron microscopic investigations of pancreas in human diabetes are very few owing to the difficulty in obtaining fresh material. Only very few studies dealing with the problem of islet amyloidosis have been published (Kawanishi

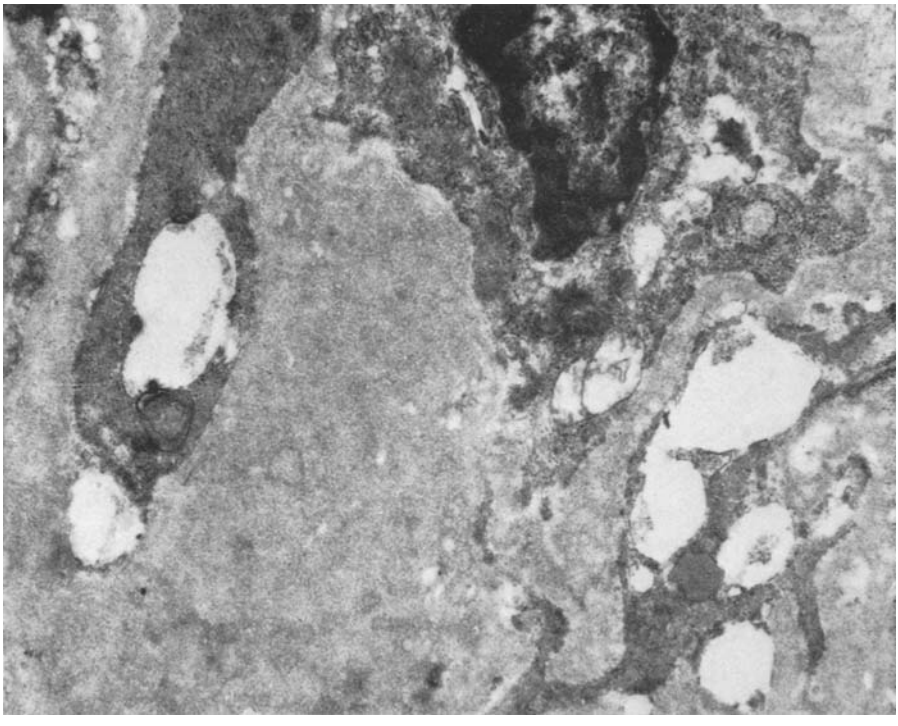

Fig. 11. Irregular, degenerating macrophages with dense cytoplasm containing vacuoles and free ribosomes. The nucleus is small and irregular. Massive amyloidosis. Diabetic patient, 9 hours after death. ×21000

et al., 1966; Yamada, 1968; Kohama *et al.*, 1969) and these have all been rather cursory.

The present investigation was carried out on autopsy material. The pancreatic tissue was fixed very soon after death. Some autolytic changes had occurred, however, and some details in the cells were therefore lost. The changes were most pronounced in the exocrine cells. As pointed out previously (Lacy, 1964), amyloid and basement membranes are preserved some time after death. In most cases, however, also endocrine cells, interstitial cells and capillaries could be studied.

It has been shown that spontaneously appearing as well as experimentally induced amyloid has an ultrastructure of non-branching fibrils about 100 Å thick (Cohen and Calkins, 1959; Caesar, 1960; Cohen, 1968). This is true also of islet amyloid (Lacy, 1964; Kawanishi *et al.*, 1966; Gueft *et al.*, 1968). Islet amyloid also has other properties common with amyloid in general. It stains with Congo red and shows green birefringence when studied in a polarization microscope under crossed polars (Ehrlich and Ratner, 1961); it shows yellow fluorescence after staining with thioflavine (Schwartz, 1965). Histochemically it has the same glycosaminoglycan pattern as other amyloid (Mowry and Scott, 1967; Vejlens and Westermark, unpublished results). However, tryptophane, which is a typical con-

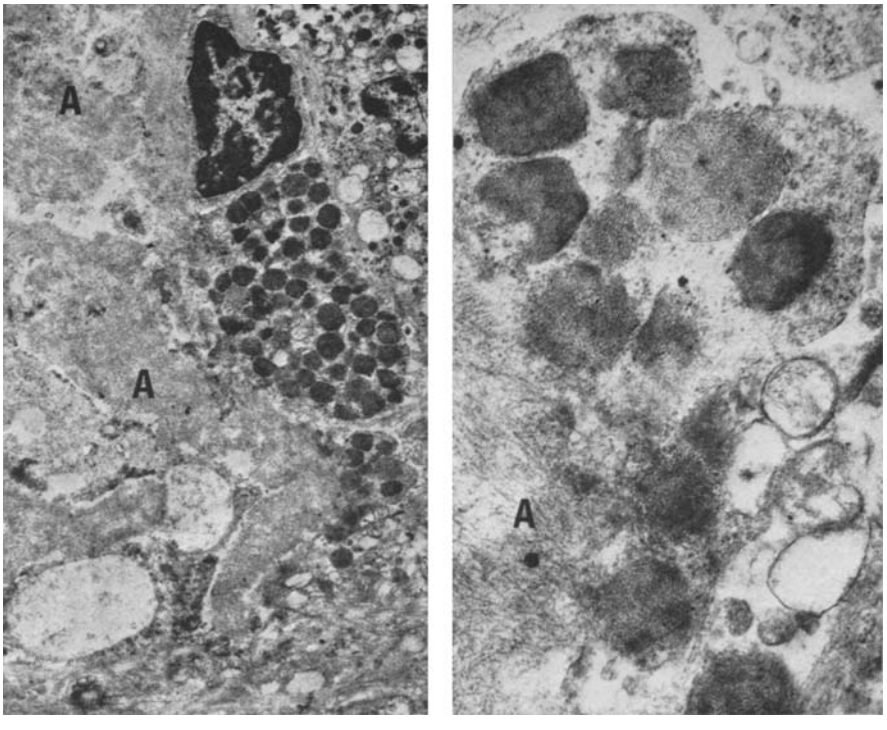

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Fig. 12a and b. Intrainsular mast cell between islet cells and amyloid. The cell is degenerating and amyloid fibrils (*A*) without distinct orientation are in contact with granules, which seem to be disintegrating. Fig. 12a is a detail of Fig. 12b. Massive amyloidosis. Diabetic patient, 4 hours after death. × 5500 and 30500

stituent of amyloid (Pearse, 1960; Cohen, 1966; Kikkawa *et al.*, 1968), cannot be demonstrated histochemically in islet amyloid (Westermark, unpublished result) or in amyloid in insulomas (Pearse *et al.*, 1972). The identity of insuloma amyloid with other amyloids has therefore been denied and it has been suggested instead to consist of C-chains of the proinsulin molecule (Pearse *et al.*, 1972).

In electron microscopic studies of the amyloid morphogenesis the amyloid fibrils have been found to be in close topographic relation to various mesenchymal cells (cf. Zucker-Franklin and Franklin, 1970). A definite orientation of the amyloid fibrils perpendicular to the cell surface and amyloid-filled invaginations in the cells have been supposed to indicate fibril formation in these cells (Cohen *et al.*, 1965; Shirahama and Cohen, 1967; Ranløv and Wanstrup, 1967, 1968; Sorenson and Bari, 1968). An active appearance of these cells has further supported this hypothesis (Shirahama and Cohen, 1967).

In this study the smallest and probably earliest deposition of amyloid occurred interstitially between capillaries and epithelial islet cells, especially β cells. The basement membranes of the β cells were sometimes indistinct and bundles of parallel amyloid fibrils were found to penetrate the basement membrane and to

run into often membrane limited pockets in the cells. When the amyloid deposition was heavier the alterations of the basement membranes and the β cells were more extensive. Sometimes similar alterations were found in α cells but never to the same extent. This close contact in combination with high orientation of the amyloid fibrils was occasionally found in macrophages and rarely in pericytes, but a lack of orientation was a much more common finding here. The result of this investigation may therefore indicate that the amyloid in the islets is a product of the β cells. There was no enlargement of the β cell nuclei (Westermark and Grimelius, 1972) and no ultrastructural signs of increased activity in these cells in any stage of islet amyloidosis. This possibly supports the view that amyloid is a degeneration product of the β cells.

Phagocytosis of amyloid by macrophages has been shown to occur (Shirahama and Cohen, 1971; Shirahama *et al.*, 1971) and the number of phagocytes was increased within the amyloid deposits in the islets. The relationship between the macrophages and the amyloid observed in the present study, with the fibrils more often than not orientated at random indicated phagocytosis rather than production. However, it could not be absolutely precluded either that the amyloid was formed by phagocytes or was deposited in the islets after having been produced elsewhere. The results of this investigation, however, contradict the conclusion of Kawanishi *et al.* (1966), who found the first amyloid precipitates in endothelial cells.

A peculiar and rather constant feature was the often rather numerous membrane limited vacuoles within the amyloid near β cells and sometimes α_2 cells. The significance of this finding is unclear. Small vesicles have previously been found in murine and human amyloid but the contents have often been electron dense (Sorenson and Bari, 1968). In the present study the vacuoles resembled the capsule of β or α cell granules. The β cells adjoining such areas were often poor in organelles including granules and the occurrence of β granules with preserved limiting membranes outside β cells in the interstitial space and in phagocytes has previously been shown (Westermark, 1972 b). Possibly the vacuoles represent remnants of β cell granules from which the contents have been released in the amyloid. Even though amyloid of insulomas is said to lack insulin activity (Lacy, 1970), insulin has been shown in islet amyloid by a fluorescent antibody technique (Berns *et al.*, 1964).

In light microscopic studies the borderline between the amyloid and the β cells has been found to be diffuse (Westermark and Grimelius, 1972). Heavy granulation of the β cells close to amyloid has also been observed (Hartroft, 1950; Westermark and Grimelius, 1972). These findings correspond well with the ultrastructural findings of bundles of amyloid fibrils entering β cells containing often densely packed granules.

Mast cells are normally found interstitially in human islets of Langerhans (Westermark, 1971 and 1972 b). They occur in an increased number in islets with amyloid deposits (Westermark, 1971). In the present study mast cells in islets with small amyloid deposits resembled other islet mast cells but more often displayed granules with granular or crystalline areas. No special topographic relation to amyloid was seen but the mast cells may have some significance in the formation of islet amyloid.

References

Ahronheim, J. H.: The nature of the hyaline material in the pancreatic islands in diabetes mellitus. Amer. J. Path. **19**, 873–882 (1943).

Arey, J. B.: Nature of the hyaline changes in islands of Langerhans in diabetes mellitus. Arch. Path. **36**, 32—38 (1943).

Beek, C. van: Amyloid-neerslag in de eilandjes van Langerhans bij diabetes mellitus. Ned. T. Geneesk. **83**, 646–654 (1939).

Berns, A. W., Owens, C. T., Blumenthal, H. T.: A histo- and immunopathologic study of the vessels and islets of Langerhans of the pancreas in diabetes mellitus. J. Geront. **19**, 179–189 (1964).

Björkman, N., Hellerström, C., Hellman, B., Petersson, B.: The cell types in the endocrine pancreas of the human fetus. Z. Zellforsch. **72**, 425–445 (1966).

Caesar, R.: Die Feinstruktur von Milz und Leber bei experimenteller Amyloidose. Z. Zellforsch. **52**, 653–673 (1960).

Cohen, A. S.: The constitution and genesis of amyloid. In: International review of experimental pathology (Richter, G. W. and Epstein, M. A., eds.), vol. 4, p. 159. New York: Academic Press 1965.

Cohen, A. S.: Preliminary chemical analysis of partially purified amyloid fibrils. Lab. Invest. **15**, 66–83 (1966).

Cohen, A. S.: High resolution ultrastructure, immunology and biochemistry of amyloid. In: Amyloidosis (Mandema, E., Ruinen, L., Scholten, J. H., and Cohen, A. S., eds.), p. 149. Amsterdam: Excerpta Medica Foundation 1968.

Cohen, A. S., Calkins, E.: Electron microscopic observations on a fibrous component in amyloid of diverse origins. Nature (Lond.) **183**, 1202–1203 (1959).

Cohen, A. S., Gross, E., Shirahama, T.: The light and electron microscopic autoradiographic demonstration of local amyloid formation in spleen explants. Amer. J. Path. **47**, 1079–1112 (1965).

De Petris, S., Karlsbad, G., Pernis, B.: Filamentous structures in the cytoplasm of normal mononuclear phagocytes. J. Ultrastruct. Res. **7**, 39–55 (1962).

Dumont, A.: Ultrastructural study of the maturation of peritoneal macrophages in the hamster. J. Ultrastruct. Res. **29**, 191–209 (1969).

Ehrlich, J. C., Ratner, I. M.: Amyloidosis of the islets of Langerhans. A restudy of islet hyalin in diabetic and nondiabetic individuals. Amer. J. Path. **38**, 49–59 (1961).

Gellerstedt, N.: Die elektive, insuläre (Para-) Amyloidose der Bauchspeicheldrüse. Zugleich ein Beitrag zur Kenntnis der „senilen Amyloidose". Beitr. path. Anat. **101**, 1–13 (1938).

Grimelius, L.: An electron microscopic study of silver stained adult human pancreatic islet cells, with reference to a new silver nitrate procedure. Acta Soc. Med. upsalien. **74**, 28–48 (1969).

Gueft, B., Ghidoni, J. J.: The site of formation and ultrastructure of amyloid. Amer. J. Path. **43**, 837–854 (1963).

Gueft, B., Kikkawa, Y., Hirschl, S.: An electron-microscopic study of amyloidosis from different species. In: Amyloidosis (Mandema, E., Ruinen, L., Scholten, J. H., and Cohen, A. S., eds.), p. 172. Amsterdam: Excerpta Medica Foundation 1968.

Hartroft, W. S.: The islets of Langerhans in man visualized by phase contrast microscopy. Proc. Amer. Diab. Ass. **10**, 46–61 (1950).

Kawanishi, H., Akazawa, Y., Machii, B.: Islets of Langerhans in normal and diabetic humans. Ultrastructure and histochemistry, with special reference to hyalinosis. Acta path. jap. **16**, 177–197 (1966).

Kikkawa, Y., Suzuki, K., Gueft, B.: Amino acid composition of urea-extracted amyloid. In: Amyloidosis (Mandema, E., Ruinen, L., Scholten, J. H., and Cohen, A. S., eds.), p. 293. Amsterdam: Excerpta Medica Foundation 1968.

Kohama, M., Moriwaki, K., Abe, H.: Electron microscopic observations of pancreatic beta cells of diabetic patients. Folia endocr. jap. **44**, 1107–1109 (1969).

Lacy, P. E.: Pancreatic beta cell. In: Aetiology of diabetes mellitus and its complications. Ciba Foundation Colloquia on Endocrinology, vol. 15, p. 75. Boston: Little, Brown & Company 1964.

Lacy, P. E.: Functional morphology of the islet cells. In: Nobel Symposium 13. Pathogenesis of Diabetes Mellitus (Cerasi, E., and Luft, R., eds.), p. 109. Stockholm: Almqvist &Wiksell 1970.

Luft, J. H.: Improvements in epoxy resin embedding methods. J. biophys. biochem. Cytol. **9**, 409–414 (1961).

Mallory, F. B.: The principles of pathologic histology. Philadelphia and London: W. B. Saunders Co. 1925.

Mowry, R. W., Scott, J. E.: Observations on the basophilia of amyloids. Histochemie **10**, 8–32 (1967).

Pearse, A. G. E.: Histochemistry. Theoretical and applied, 2nd ed. London: J. & A. Churchill Ltd. 1960.

Pearse, A. G. E., Ewen, S. W. B., Polak, J. M.: The genesis of apudamyloid in endocrine polypeptide tumours: histochemical distinction from immunamyloid. Virchows Arch. Abt. B **10**, 93–107 (1972).

Ranløv, P., Wanstrup, J.: Ultrastructural investigations on the cellular morphogenesis of experimental mouse amyloidosis. Acta path. microbiol. scand. **71**, 575–591 (1967).

Ranløv, P., Wanstrup, J.: Electron-microscopic demonstration of intracellular amyloid in experimental mouse amyloidosis. In: Amyloidosis (Mandema, E., Ruinen, L., Scholten, J. H., and Cohen, A. S., eds.), p. 74. Amsterdam: Excerpta Medica Foundation 1968.

Reynolds, E. S.: The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. **17**, 208–212 (1963).

Schwartz, P.: Über Amyloidose des Gehirns, der Langerhansschen Inseln und des Herzens alter Personen. Zbl. allg. Path. path. Anat. **108**, 169–187 (1965).

Shirahama, T., Cohen, A. S.: A Congo red staining method for epoxy-embedded amyloid. J. Histochem. Cytochem. **14**, 725–729 (1966).

Shirahama, T., Cohen, A. S.: Fine structure of the glomerulus in human and experimental renal amyloidosis. Amer. J. Path. **51**, 869–911 (1967).

Shirahama, T., Cohen, A. S.: Lysosomal breakdown of amyloid fibrils by macrophages. Amer. J. Path. **63**, 463–485 (1971).

Shirahama, T., Cohen, A. S., Rodgers, O. G.: Phagocytosis of amyloid: *In vitro* interaction of mouse peritoneal macrophages with human amyloid fibrils and their accelerated uptake after dye binding. Exp. molec. Path. **14**, 110–123 (1971).

Sorenson, G. D., Bari, W. A.: Murine amyloid deposits and cellular relationships. In: Amyloidosis (Mandema, E., Ruinen, L., Scholten, J. H., and Cohen, A. S., eds.), p. 58. Amsterdam: Excerpta Medica Foundation 1968.

Sorenson, G. D., Heefner, W. A., Kirkpatrick, J. B.: Experimental amyloidosis. II. Light and electron microscopic observations of liver. Amer. J. Path. **44**, 629–644 (1964).

Teilum, G.: Pathogenesis of amyloidosis. The two-phase cellular theory of local secretion Acta path. microbiol. scand. **61**, 21–45 (1964).

Watson, M. L.: Staining of tissue sections for electron microscopy with heavy metals. J. biophys., biochem. Cytol. **4**, 475–478 (1958).

Westermark, P.: Mast cells in the islets of Langerhans in insular amyloidosis. Virchows Arch. Abt. A **354**, 17–23 (1971).

Westermark, P.: Quantitative studies of amyloid in the islets of Langerhans. Upsala J. Med. Sci. **77**, 91–94 (1972a).

Westermark, P.: Ultrastructure of capillaries and interstitial cells in human pancreatic islets. Upsala J. Med. Sci., in press, 1972b.

Westermark, P., Grimelius, L.: The pancreatic islet cells in insular amyloidosis in human diabetic and non-diabetic adults. In preparation, 1972.

Yamada, Y.: Pathologic study on amyloidosis. Amyloidosis of the islets of Langerhans in diabetes mellitus. Bull. Yamaguchi med. Sch. **15**, 227–250 (1968).

Zucker-Franklin, D., Franklin, E. C.: Intracellular localization of human amyloid by fluorescence and electron microscopy. Amer. J. Path. **59**, 23–41 (1970).

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