

Cytochemical study of the involvement of cell organelles in formation and accumulation of fibrillar amyloid in the pancreas of NOR β transgenic mice

**D.H. Dobrogowska¹, A.W. Vorbrodt¹, J. Wegiel¹, K-C. Wang¹, M. Shoji²,
C. Mondadori³, G. Polatis³, A. Giovanni³ and H.M. Wisniewski¹**

¹New York State Office of Mental Retardation and Developmental Disabilities, Institute for Basic Research in Developmental Disabilities, Staten Island, New York, USA, ²Gunma University, School of Medicine, Maebashi, Gunma, Japan and

³Aventis Pharmaceuticals, Bridgewater, NJ, USA

Summary. Phosphatase ultrastructural cytochemistry was used to evaluate the participation of cytoplasmic organelles in the accumulation of fibrillar amyloid β (A β) in exocrine acinar cells and in macrophages of the pancreas of transgenic mice overexpressing a carboxy-terminal fragment of A β protein precursor (A β PP). Nucleoside diphosphatase (NDPase) and glucose-6-phosphatase (G6Pase) were used as cytochemical markers of the endoplasmic reticulum (ER), thiamine pyrophosphatase (TPPase) as a marker of the Golgi apparatus (GA), and acid phosphatase (AcPase) as a marker of lysosomes. Monoclonal antibody 4G8 raised against the 17-24 aa sequence of human A β protein was used for immunogold localization of fibrillar A β . The results of this study indicate that the formation of A β in acinar cells occurs directly in the vacuolar areas of the rough ER (RER) without evident participation of the elements of the GA, whereas an intimate structural relation with primary lysosomes suggests their role in modification or digestion of the deposited amyloid.

In macrophages, fibrillar amyloid was present in numerous cytoplasmic vacuoles located frequently in close proximity to flattened saccules of the ER. This structural pattern revealed similarity to that observed previously in microglial cells producing fibrillar PrP amyloid in scrapie-infected mice and A β in brains of human elderly patients and in Alzheimer's type brain pathology.

Key words: Amyloid- β , Pancreas, Transgenic mice, Cytoplasmic organelles, Cytochemical markers

Introduction

The cellular mechanisms of amyloid formation and deposition are not fully understood. In brains of subjects with Alzheimer's disease (AD) and unconventional viral infections (e.g. Gerstmann-Strausler syndrome, Kuru, Creutzfeldt-Jacob disease), the amyloid is deposited extracellularly, mainly in plaques and in the wall of blood vessels (Wisniewski et al., 1982). Similar pattern of the distribution of amyloid deposits in the central nervous system (CNS) has been noted in scrapie-infected mice (Wisniewski et al., 1986, 1990). Ultrastructural and cytochemical observations suggest that the amyloid fibrils deposited mostly in plaques emerge from the sacs of the endoplasmic reticulum of microglial cells (Wisniewski et al., 1982, 1986, 1990, 1991, 1992).

There are, however, contradictory opinions suggesting that the amyloid is produced inside the neurons, and after externalization accumulates extracellularly (Powers, 1986; Cork et al., 1990; Pappolla et al., 1991; Probst et al., 1991; Price et al., 1998). Assuming that the production and/or accumulation of fibrillar amyloid takes place in the similar manner as that of other proteins, one can anticipate that some related cell organelles are engaged in this process. Although at the ultrastructural level the particular organelles can be morphologically recognized, one can encounter occasionally such a complex structural interrelations between them that make the interpretation of obtained pictures difficult. The use of cytochemical markers of cell organelles can facilitate getting insight into their structural-functional relations and involvement in formation and deposition of amyloid fibrils.

Taking this into consideration we applied cytochemical markers of some cytoplasmic organelles related to the process of protein synthesis in accordance

Offprint requests to: D.H. Dobrogowska, New York State Office of Mental Retardation and Developmental Disabilities, Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Island, New York 10314, USA. Fax: 718-982-6346

with suggestions presented and discussed in details by Novikoff and Essner (1962) and Novikoff and Novikoff (1972). In this work the following markers of cytoplasmic organelles were used: nucleoside diphosphatase (NDPase) as a marker of the endoplasmic reticulum (ER) and Golgi apparatus (Novikoff, 1976; Novikoff and Goldfischer, 1961); glucose-6-phosphatase (G6Pase) as an additional marker of the ER (Leskes et al., 1971); thiamine pyrophosphatase (TPPase) as a marker of the Golgi apparatus (Novikoff and Goldfischer, 1961); acid phosphatase (AcPase) as a marker of lysosomes and related structures including Golgi-endoplasmic reticulum-lysosomes (GERL) complex (Novikoff, 1963). The scheme presented by Palade (1975) indicates that all these cytoplasmic structures are presumably engaged in the intracellular process of protein synthesis.

The purpose of our present study was to determine whether or not the mentioned organelles are involved in the formation and accumulation of fibrillar amyloid β (A β) in the exocrine cells and in monocytes/macrophages of the pancreas of NOR β transgenic mice. These mice overexpressing a carboxy-terminal fragment of amyloid β protein precursor (A β PP) constitute a unique and valuable animal model for studying of intracellular production and accumulation of A β peptides (Kawarabayashi et al., 1996; Wegiel et al., 2000). In these mice the highest expression of protein encoded by transgene is seen in pancreas and only in pancreas fibrillar A β accumulates in cytoplasmic vacuoles (Shoji et al., 1998).

Materials and methods

For cytochemical detection of NDPase, TPPase and AcPase activities, transgenic mice NOR β /128-44/C57/618x1+, 14 month-old, were perfused at room temperature, under Nembutal (sodium barbiturate) anesthesia, with the fixative containing 2% formaldehyde (freshly prepared from paraformaldehyde) and 1% glutaraldehyde in 0.2M cacodylate buffer, pH 7.4, followed by immersion fixation in ice cold fixative (up to 2 hrs). After fixation, the tissue samples were rinsed overnight in cold (4 °C) 0.05M cacodylate buffer containing 0.15M sucrose. The cytochemical localization of all marker enzymes under consideration was originally established for cytoplasmic organelles of the liver cell (Novikoff and Essner, 1962; Leskes et al., 1971), therefore in addition to the pancreas liver tissue samples were taken serving as a positive control. For cytochemical detection of G6Pase activity the tissue samples were immersion-fixed for 10 min in ice-cold fixative containing 2% formaldehyde, 0.5% glutaraldehyde, 0.006M glucose-6-phosphate, 0.002 M CaCl₂, 0.15M sucrose in 0.05M cacodylate buffer, pH 7.2. They were rinsed overnight in ice-cold 0.05M cacodylate buffer, pH 7.2, containing 0.001M glucose-6-phosphate and 0.2M sucrose (Manns, 1968; Leskes et al., 1971). The following day sections of 35-40 μ m

thickness were prepared with the tissue sectioner (chopper) Sorvall TC-2 (Du Pont).

Freely floating sections were incubated, at 37 °C in a Dubnoff shaking water bath for detection of the activity of the following enzymes:

1) TPPase according to Novikoff and Goldfischer (1961), in a medium containing 25 mg thiamine pyrophosphate sodium salt (Sigma Chemical Co., St. Louis, Mo.); 9.4 ml of distilled water; 10.0 ml of 0.2M Tris-maleate buffer, pH 7.4; 0.6 ml of 0.2M manganese chloride; 2.5 ml of 0.033M lead nitrate and 2.5 ml of 1M sucrose. The incubation time was 80 min.

2) NDPase in a medium similar to that used in our previous studies (Vorbodt and Wisniewski, 1982; Wisniewski et al., 1991), containing 25 mg of inosine-5'-diphosphate (IDP) sodium salt (final concentration 0.002M); 10 ml of 0.2M Tris-maleate buffer, pH 7.4; 0.6 ml of 0.2M manganese chloride; 2.5 ml of 1M sucrose; 9.4 ml of distilled water and 2.5 ml of 0.033M lead nitrate. The medium had final pH 7.2; the tissue was incubated 70 minutes.

3) AcPase was detected by the application of two different methods: a) the medium prepared according to Barka and Anderson (1962) contained 45 mg of sodium β -glycerophosphate; 20 ml of 0.2M Tris-maleate buffer, pH 5; 4 ml of 0.033M lead nitrate; 5 ml of 1M sucrose and 21 ml of distilled water. Specimens were incubated for 50 minutes in previously filtered medium; b) the medium prepared according to Novikoff (1963) consisted of 50 mg of cytidine-5'-monophosphate (CMP); 25 ml of 0.1M acetate buffer, pH 5; 5 ml of 0.033M lead nitrate; 7.5ml of 1M sucrose and 12.5 ml of distilled water. The incubation time in this medium was 50 minutes.

4) G6Pase activity was detected according to Leskes et al. (1971). The medium contained 28 mg of glucose-6-phosphate (sodium salt), 12.5 ml of 0.2M Tris-maleate buffer, pH 6.6; 25 ml of distilled water; 7.5 ml of 1M sucrose, 5 ml of 0.033M lead nitrate. The incubation was performed at 30 °C for 40 min.

In control media the phosphate esters were omitted. After incubation, the sections were washed in 7.5% sucrose, postfixed for 1 h in ice-cold 1% OsO₄ in 0.1M cacodylate buffer, pH 7.2, containing 4.5% sucrose, and finally stained *en block* (in refrigerator) with 0.5% uranyl acetate, pH 5. Next day the sections were dehydrated in ethanol and embedded in Spurr low viscosity medium. Ultrathin sections were cut on Sorvall MT 5000 microtome, stained with lead citrate and examined in a Hitachi 7000 electron microscope.

Some incubated and non-incubated sections were processed for immuno-cytochemical detection of fibrillar amyloid- β as follows. Ultrathin sections collected on nickel grids were immunolabeled with monoclonal antibody 4G8 (mAb 4G8), diluted 1:40 in PBS containing 0.5% ovalbumin, raised against the 17-24 amino acid (aa) sequence of human A β protein (Kim et al., 1988). After overnight incubation in a refrigerator and washing with PBS, the sections were exposed to

secondary antibodies (goat anti-mouse) labeled with colloidal gold of 20 nm diameter diluted 1:20 with PBS (AuroProbe EM, GAM IgG G20, Amersham Life Science) for 1 h at room temperature, washed and stained with uranyl acetate and lead citrate (Wegiel et al., 2000).

Results

A) Cytochemical and immunocytochemical observations of exocrine (acinar) pancreatic cells

The endoplasmic reticulum (ER).

In parenchymal liver cells the cytochemical reaction for NDPase activity is localized solely in the flattened saccules (cisternae) of rough and smooth ER, and in the nuclear envelope (Fig. 1). In the exocrine pancreas of NOR β transgenic mice, these cytoplasmic structures remain unstained, whereas electron dense deposits of the reaction product (r.p.) for NDPase appear in the inner (trans) elements of the Golgi apparatus (GA) and in or between plasma membranes of the adjacent cells (Fig. 2).

The distribution of fibrillar A β deposits reveal their very intimate relation to membranes of the ER. The deposits of A β immunolabeled with 4G8 antibodies are present in relatively large vacuoles or vacuole-like spaces located among the flattened saccules of the ER (Fig. 3, A). It seems that these vacuolar structures are not enclosed with well-defined and continuous membranous envelope. They are, however, surrounded mostly by rough ER membranes (RER).

Cytochemical reaction for G6Pase, used as an another enzymatic marker of the ER, appears to be

strongly positive in membranes of the RER and in nuclear envelope of the parenchymal liver cells (Fig. 4), but is absent in the similar organelles of the exocrine pancreatic cells (Fig. 5).

The Golgi apparatus (GA)

The cytochemical reaction for TPPase in exocrine pancreas cells is localized in the innermost (trans) elements of the GA and in or on the plasma membrane of these cells (Fig. 6). Occasionally, electron-dense deposits of the r.p. for TPPase appear in some cytoplasmic vacuoles containing fibrillar A β . These vacuoles are usually small or medium-sized (Fig. 7, arrowhead and arrows). In other, usually larger vacuoles containing fibrillar A β , the scanty and vestigial precipitates of the r.p. are sometimes present (Fig. 7, A). In a majority of larger vacuoles which contain immunoreactive A β deposits no r.p. for TPPase appears (Fig. 8, A).

Whereas a close structural relation exists between fibrillar amyloid-containing vacuoles and membranes of flattened sacs of the RER (Figs. 3, 7 and 8), no relation of these vacuoles to the GA elements can be noted. In contrast, the inner, mature face of the GA seems to be in structural contact with condensing vacuoles and zymogen granules (Figs. 2, 3 and 8).

The lysosomes and related organelles

The AcPase activity in pancreatic acinar cells is distributed unevenly in various lysosomal structures. Several small vesicles containing dense deposits of the r.p., representing presumably primary lysosomes, are situated frequently in close proximity to larger vacuoles containing fibrillar, amyloid-like material (Figs. 9, 10).

Fig. 1. A portion of a liver cell after incubation for cytochemical demonstration of NDPase activity. The deposits of the reaction product (r.p.) are localized solely in the flattened sacs of the rough endoplasmic reticulum (RER, arrows), and in the nuclear envelope (arrowheads). No r.p. appears in the mitochondria (M), cell nucleus (N), nucleolus (Nu), and in the sinusoidal endothelial cell (E). Bar: 1 μ m. x 15,000

Fig. 2. Cytochemical reaction for NDPase activity in pancreatic acinar cells of NOR β transgenic mouse. The r.p. appears in the innermost (trans) elements of the Golgi apparatus (arrows) but is absent in the sacs of the RER. Condensing vacuoles (C) are located in close apposition to the inner elements of the Golgi apparatus, whereas numerous mature secretion (zymogen) granules (Z) are gathered in the apical portion of the cell facing the acinar lumen (L). Some r.p. is deposited between adjacent cells (curved arrow). No reaction for NDPase appears in the vacuoles containing fibrillar amyloid deposits (arrowheads), which are located in the basal portion of the acinar cell filled up by flattened sacs of the RER. Bar: 1 μ m. x 10,000

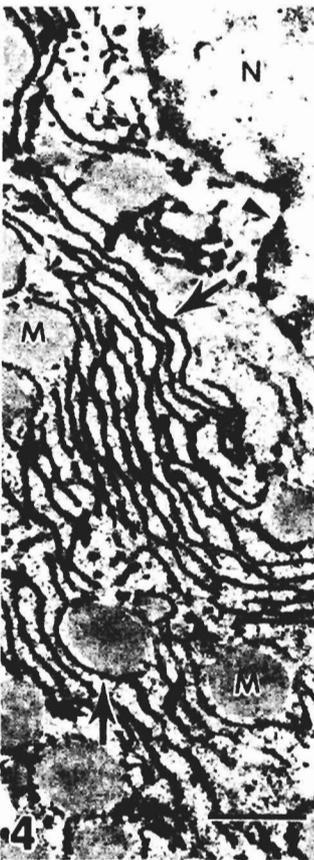
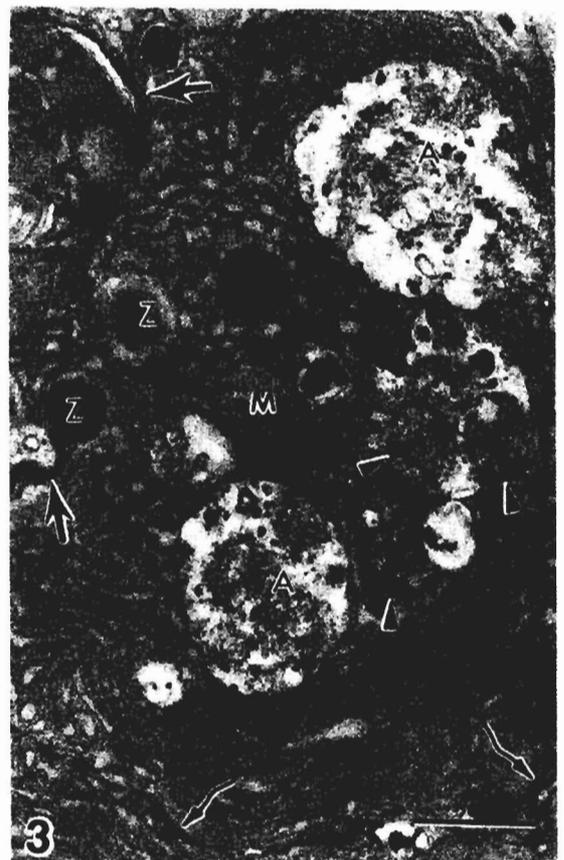
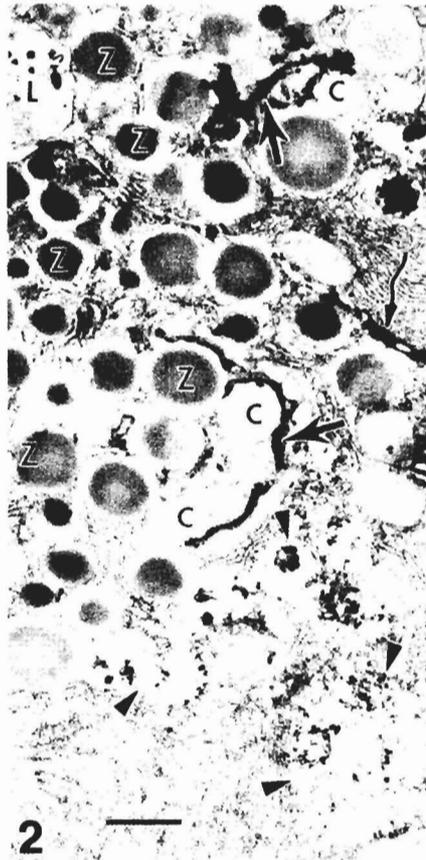
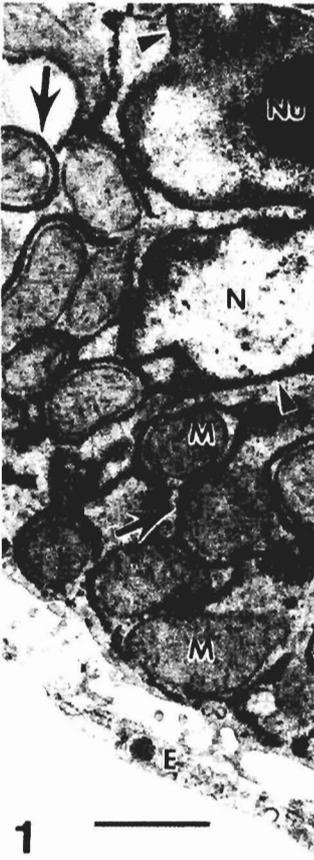
Fig. 3. A portion of a pancreatic acinar cell shown after incubation for cytochemical visualization of NDPase activity and immunocytochemical detection of A β deposits. The r.p. for NDPase is localized in the trans (inner) elements of the Golgi apparatus (arrows) and between adjacent acinar cells (curved arrows). Immunogold reaction for amyloid is present inside the vacuoles (A), and in perivacuolar areas of various sizes and irregular shape, the outlines of which are intermixed with the ER membranes (arrowheads). Bar: 1 μ m. x 16,000

Fig. 4. A portion of a liver cell (as a positive control) incubated for the demonstration of G6Pase activity. The electron-dense r.p. is localized solely in the flattened sacs of the ER (arrows) and in some segments of the nuclear envelope (arrowhead). No reaction appears in the mitochondria (M) or in the cell nucleus (N). Bar: 1 μ m. x 12,500

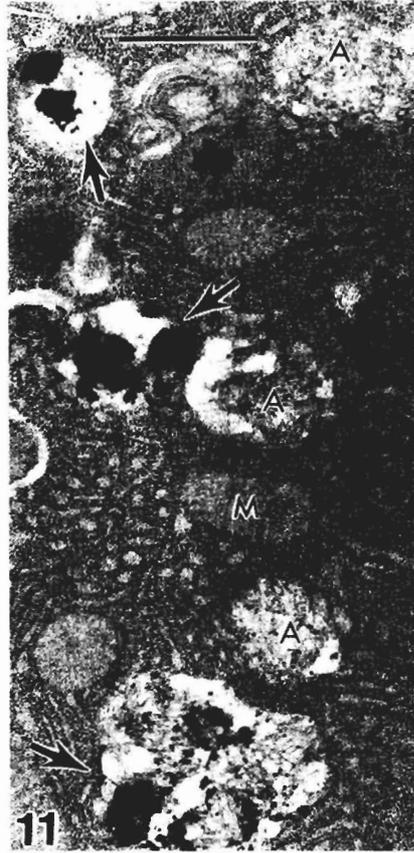
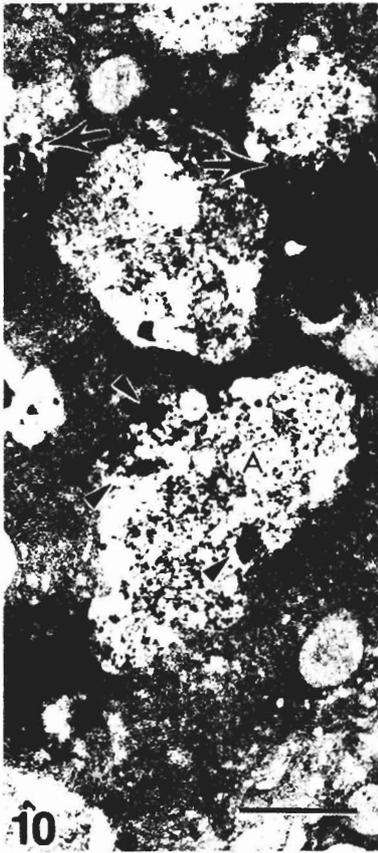
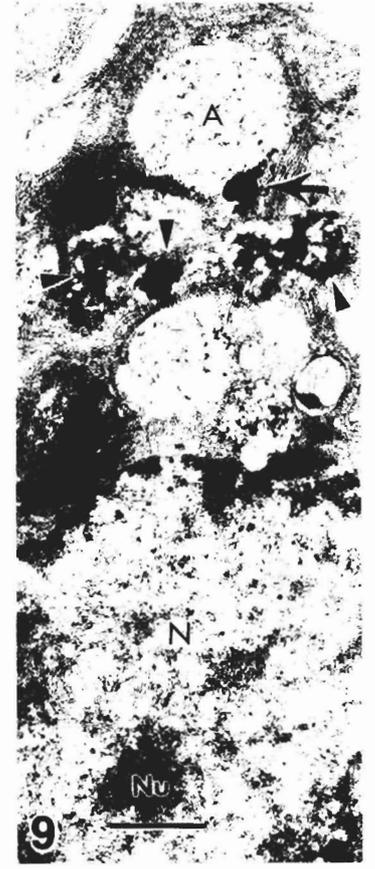
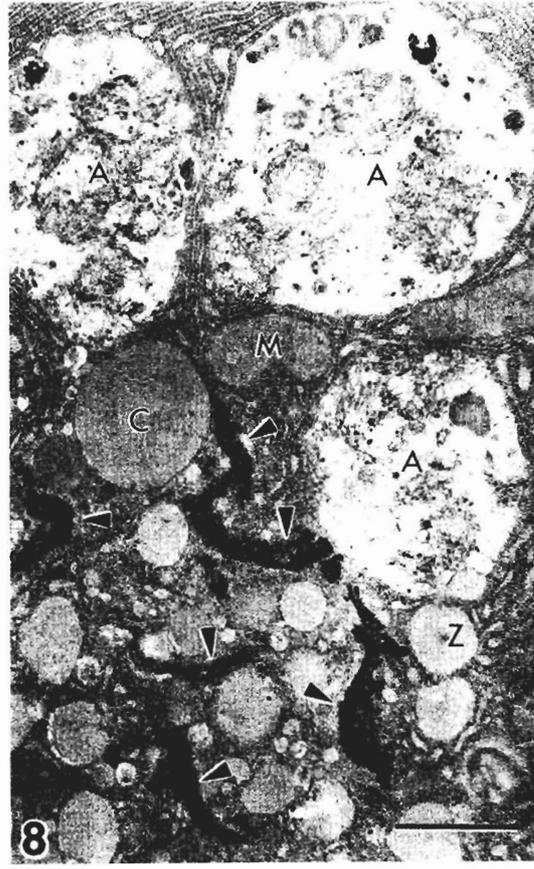
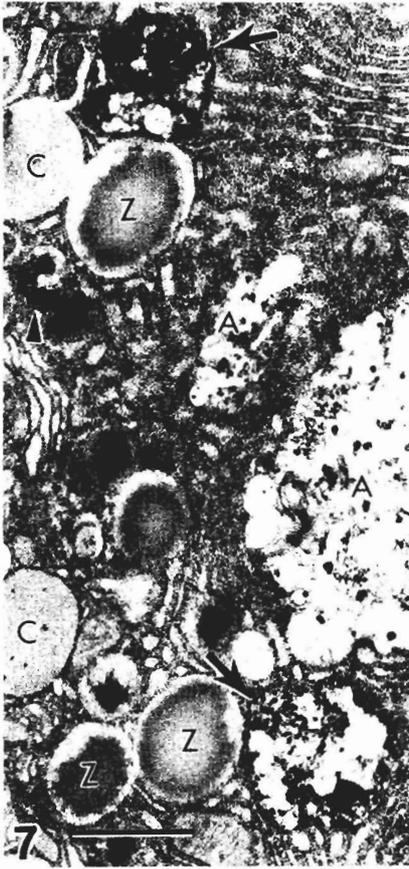
Fig. 5. A portion of a pancreatic acinar cell incubated for cytochemical detection of G6Pase activity. No r.p. deposits appear in the flattened sacs of the ER, in mitochondria (M) and in fibrillar amyloid-containing vacuoles (A). Bar: 1 μ m. x 14,000

Fig. 6. A portion of a pancreatic acinar cell with cytochemically demonstrated TPPase activity and immunocytochemically labeled amyloid deposits. The r.p. for TPPase is localized in the innermost elements of the Golgi apparatus (arrows) and also between adjacent acinar cells (curved arrow). Immunosignals for amyloid are located inside the vacuoles tightly associated with the membranes of the RER (A) or in enlarged or swollen sacs of the ER (arrowhead). Bar: 1 μ m. x 15,000

Cell organelles and amyloid formation



Cell organelles and amyloid formation



Cell organelles and amyloid formation

Fig. 7. In this portion of the pancreatic acinar cell the r.p. for TPPase is present in a small (arrowhead) and in larger vacuoles (arrows) which show some structural similarity to amyloid-containing vacuoles (A). Condensing vacuoles (C) and zymogen granules (Z) are free from the r.p. Bar: 1 μ m. x 6,000

Fig. 8. In this portion of the pancreatic acinar cell the TPPase activity is located in the extensively ramified tubular and cisternal portions of the Golgi apparatus (arrowheads). Immunogold reaction for amyloid is restricted to three large vacuoles (A) which are in close apposition to (are placed in juxtaposition to) the membranes of RER. Neither condensing vacuoles (C), zymogen granules (Z) nor mitochondria (M) are labeled. Bar: 1 μ m. x 16,000

Fig. 9. A portion of a pancreatic acinar cell after incubation in a cytochemical medium for detection of AcPase activity. Electron-dense r.p. is localized in relatively small primary lysosomes, the one of which (arrow) is apparently attached to a larger vacuole containing fibrillar amyloid (A). There are also irregularly shaped vacuoles (endosomes?) containing less dense deposits of the r.p. (arrowheads). N: nucleus; Nu: nucleolus. Bar: 1 μ m. x 12,000

Fig. 10. Another portion of a pancreatic acinar cell incubated for cytochemical localization of AcPase activity. The AcPase-positive vesicles (primary lysosomes) are fused with vacuoles presumably containing amyloid fibrils (arrows). One larger amyloid-containing vacuole (A) shows also deposits of the r.p. for AcPase (arrowheads). x 15,000

Fig. 11. A portion of a pancreatic acinar cell after incubation for cytochemical detection of AcPase activity and for immunogold labeling of amyloid deposits. There are three amyloid-containing (immunogold-labeled) vacuoles which do not contain the r.p. for AcPase (A), and three vacuoles which contain both immunogold-labeled amyloid and deposits of the r.p. for AcPase (arrows). M: mitochondria. Bar: 1 μ m. x 18,000

Fig. 12. The same section as presented in Fig. 11 shown under higher magnification. In this synthetic portion of the acinar cell filled up with arrays of the RER cisternae two immunogold-labeled vacuoles contain only amyloid (arrowheads), whereas three other vacuoles contain both immunolabeled amyloid and deposits of the r.p. for AcPase (arrows). M: mitochondria. Bar: 1 μ m. x 20,000

Some of these large vacuoles are labeled with unevenly dispersed precipitates of the r.p. for AcPase (Figs. 9, 10, arrowheads). In sections incubated in both cytochemical and immunocytochemical media many vacuoles contain only gold-labeled A β , whereas other vacuoles contain both gold-labeled A β and deposits of the r.p. for AcPase activity of various density (Figs. 11, 12).

B) Cytochemical and immunocytochemical observations of macrophages in the pancreas

All macrophages infiltrating the stroma and parenchyma of the pancreas of transgenic mice contain numerous cytoplasmic vacuoles filled up with fibrillar material immunolabeled with mAb 4G8.

In many macrophages the cytochemical reaction for NDPase activity appears in short segments of a thin, flattened sacs of the ER squeezed between amyloid-containing vacuoles (Fig. 13). In some macrophages the

r.p. for NDPase activity appears in short, thin segments of the inner saccules of the GA only (Fig. 14, arrows).

Cytochemical reaction for TPPase activity in the GA of macrophages is weak and inconspicuous, probably because of small size of this organelle. In some macrophages the staining reaction for TPPase of various intensity appears in cytoplasmic structures which show irregular shape and contain amyloid-like material (Fig. 15).

The reaction for AcPase activity in a majority of macrophages is of low and variable intensity. The electron-dense precipitates of the r.p. are usually scattered among fibrillar amyloid-like material present inside large cytoplasmic vacuoles (Fig. 16). These vacuoles of variable and irregular shapes bear a resemblance to the above-described TPPase-positive structures and can be defined as endosomes.

The immunogold reaction for A β is localized in several cytoplasmic vacuoles of some macrophages

Fig. 13. Perivascular macrophage infiltrating the pancreatic stroma incubated for cytochemical detection of NDPase activity. Electron-dense deposits of the r.p. appear in some segments of flattened saccules of the endoplasmic reticulum (arrowheads) squeezed (interposed) between numerous fibrillar amyloid-containing vacuoles (A). Bar: 1 μ m. x 10,000

Fig. 14. Another macrophage infiltrating the parenchyma of the pancreas after cytochemical incubation for NDPase and immunocytochemical localization of amyloid deposits. Thin precipitates of the r.p. for NDPase appear only in a few innermost Golgi saccules (arrows), whereas numerous amyloid-containing vacuoles are labeled with immunogold only (arrowheads). N: cell nucleus. Bar: 1 μ m. x 14,000

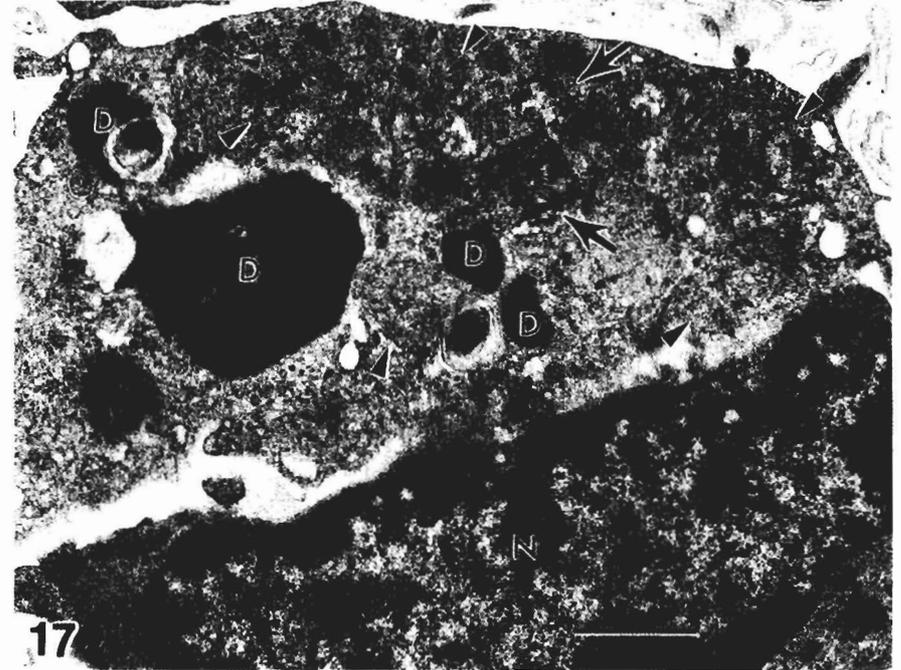
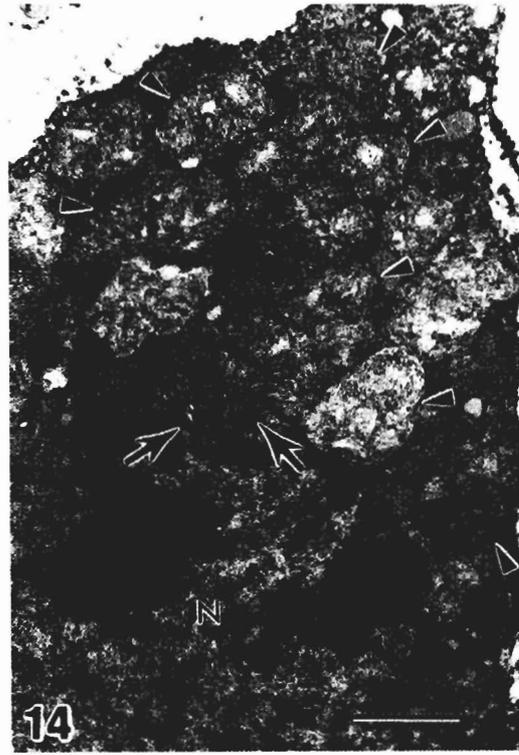
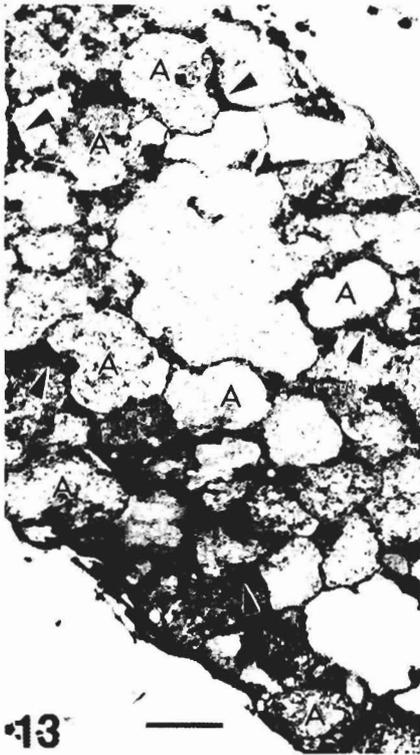
Fig. 15. Perivascular macrophage after incubation in cytochemical medium for demonstration of TPPase activity. Precipitates of the r.p. of various density appear in several amyloid-containing cytoplasmic vacuoles (arrowheads). The Golgi apparatus is not visible in the sectioned part of the cell. Bar: 1 μ m. x 14,000

Fig. 16. Another perivascular macrophage after incubation for cytochemical demonstration of AcPase activity. Unevenly scattered small precipitates of the r.p. (arrowheads) appear among amyloid fibrils located inside the cytoplasmic vacuoles (endosomes). N, cell nucleus, G, Golgi apparatus. Bar: 1 μ m. x 16,000

Fig. 17. The macrophage infiltrating the pancreatic stroma after incubation for cytochemical demonstration of AcPase activity and immunocytochemical localization of fibrillar A β . Immunogold labeling is present in several cytoplasmic areas and in vacuole-like structures (arrowheads), whereas r.p. for AcPase is present in two vacuoles only (arrows). In this macrophage few dense body-like structures (D), which do not show the cytochemically detectable AcPase activity, are located in the cytoplasm. N: cell nucleus. Bar: 1 μ m. x 16,000

infiltrating the stroma of the pancreas. Some of these vacuoles contain also electron-dense precipitates of the r.p. for AcPase activity (Fig. 17, arrows). The electron-dense cytoplasmic bodies (dense bodies?) present inside these cells neither show positive reaction for AcPase activity nor contain immuno-reactive A β (Fig. 17, D).

Occasionally observed appearance of precipitates of the r.p. in lysosome-like (Fig. 7) or endosome-like (Fig. 15) structures after incubation of tissue sections in TPPase medium, results probably from the hydrolysis of TPP by unspecific phosphatase (AcPase) normally present in these organelles, as shown in Fig. 16.



Discussion

Our observations revealed that the application of ultrastructural cytochemistry for visualization of marker enzymes of pancreatic acinar cell organelles of transgenic mice was of limited value for evaluating their participation in formation and accumulation of fibrillar A β . This limitation relates mostly to both NDPase and G6Pase activities, which were excellent markers of the ER in parenchymal liver cells (Figs. 1, 4), whereas appeared to be less valuable in exocrine pancreatic cells. The role and involvement of the ER in these cells could be evaluated only on the basis of structural interrelation of the ER with amyloid deposits.

The localization of NDPase activity in the acinar cells was identical to that of TPPase, indicating that both enzymes could be considered efficient cytochemical markers of the GA, which is consistent with earlier observations of Novikoff et al. (1977, 1978) and of Hand and Oliver (1977). On the other hand, cytochemically demonstrable AcPase activity gave most valuable informations on the alleged involvement of lysosomes and related organelles in elaboration and deposition of fibrillar A β .

The pancreatic acinar cell is highly polarized, with extensive arrays of the RER cisternae occupying a large basal portion of the cytoplasm between cell nucleus and basolateral plasma membrane, defined as a synthetic pole (Palade, 1975; Novikoff et al., 1977). The apical portion (secretory pole) contains condensing vacuoles and zymogen granules, the number of which varies with the functional state of the acinar cell. When many granules are present, the GA is located both above and lateral to the nucleus, and its mature face (inner or trans elements) is in close apposition to condensing vacuoles. AcPase-positive primary lysosomes and smooth-surfaced (rigid) lamellae of GERL are located in close proximity to GA, between the nucleus and secretory pole of the cell (Novikoff et al., 1977, 1978).

The polarization of the pancreatic acinar cell is considered to be a structural reflection of the extensive protein synthesis and secretory process which, according to Palade (1975), occurs in six steps: synthesis, segregation, intracellular transport, concentration, storage and ultimately discharge of the secretory product into the acinar lumen. In all these steps the cytoplasmic organelles, mainly ER, GA, and AcPase-positive structures of GERL, as postulated by Novikoff et al. (1977, 1978), are involved.

These consecutive steps of protein synthesis and secretion for extracellular use mostly (i.e. for export) are typical for the exocrine pancreas. Thus, our main attention was focused on the structural relationship between this well-ordered secretory machinery and disturbing intruders represented by fibrillar A β , which appears in the pancreas of transgenic mice.

Our findings revealed that although the distribution of amyloid deposits was not related to the cell polarity, in most instances they were in rather close or even

intimate structural relation to the ER membranes. No evidence of close structural relation to cis or trans elements of the GA was noted. There was also no tendency to the transfer of amyloid deposits to the apical part of the cell (secretory pole) or their discharge into the acinar lumen.

These observations strongly suggest that the fibrillar A β is assembled directly in the cisternae of the RER without involvement of the GA and with omission of consecutive steps of the secretory process described above. It seems interesting that according to Palade (1975) and Hand and Oliver (1977), the protein synthesis can also occur in some cell types in a more simple way consisting in the secretion of the product by budding of the ER saccules and its transfer to the vacuoles. One can assume that such a mechanism operates in the acinar cell of the pancreas of transgenic mice producing fibrillar A β .

The activity of AcPase demonstrated in the sections of exocrine pancreas of transgenic mice (Figs. 9-12) shows close structural relation of the primary lysosomes and the A β -containing vacuoles. The interpretation of this finding can be twofold:

(i) The deposits of the fibrillar amyloid are treated as an abnormal, foreign material and are sequestered and degraded by lysosomal enzymes in a manner similar to that observed in autophagic vacuoles. The synthesis or deposition of fibrillar A β seems to occur in vacuolar spaces scattered among the RER cisternae without evident participation of other organelles. It suggests that this process bear the feature of *sui generis* aberrant or abortive synthetic pathway. As a result, the lysosomal reaction occurs which can be considered a defensive action the purpose of which is to eliminate (by digestion) this product, resulting from artificial introduction of a foreign genetic information. Such a reaction resembles the destruction of the islet amyloid polypeptide by macrophages (Koning et al., 1998).

(ii) The lysosomes participate in the process of production of A β in pancreatic acinar cells of transgenic mice, as recently postulated by Kawarabayashi et al. (1997). Haass et al. (1992) also presented data suggesting that A β precursor protein can be targeted from the cell surface to the endosomal-lysosomal compartments where A β peptides appear as a products of proteolysis. Their version, however, is difficult to accept because in pancreatic acinar cells we did not notice any structural signs suggesting the process of endocytosis of material from the cell surface and/or transport of the endocytosed material to the endosomes or lysosomes. We also observed the immunolabeled A β in the cytoplasmic vacuoles, which did not show positive reaction for AcPase. It indicates that A β was present inside the vacuoles before they fused with primary lysosomes, which discharged own digestive enzymes, including cytochemically detected AcPase, into their interior.

Our observations of macrophages infiltrating the stroma and occasionally the parenchyma of the pancreas

of transgenic mice indicate that cytochemical reaction for NDPase can be considered the enzymatic marker of the ER in a majority of these cells. Several macrophages revealed very close structural relation between NDPase-positive segments of the ER and A β -containing vacuoles (Fig. 13). The differences observed are probably related to different physiological activity of particular cells at the moment of their fixation.

It seems noteworthy to mention our previous observations indicating a close structural relation between cisternae of the ER and fibrillar amyloid deposits in microglial cells located at the periphery or inside the amyloid plaques in the brains of scrapie-infected mice (Wisniewski et al., 1990) and elderly human patients (Wisniewski et al., 1991). These observations prompted us to express opinion that microglial cells, which are homologous to macrophages, participate in synthesis and deposition of fibrillar amyloid (Wegiel and Wisniewski, 1999; Wisniewski et al., 1992).

The presence of numerous cytoplasmic vacuoles containing fibrillar A β in pancreatic macrophages of transgenic mice may indicate that A β was endocytosed or phagocytosed by these cells, or was synthesized inside their cytoplasm. If the amyloid material was phagocytosed, it should be deposited in AcPase-rich endosomes or phagosomes and should show various stages of decomposition by digestive enzymes paralleled by uneven diminution of the size of amyloid-containing vacuoles. But the cytochemical reaction for AcPase was weak and appeared only in a few cells.

On the other hand, the presence of fibrillar amyloid-containing vacuoles in close apposition to some segments of the ER suggests that they are the site of the A β production. Such a possibility is consistent with Palade's opinion (1975) that the process of protein synthesis in macrophages differs from that observed in exocrine pancreas cells and occurs without the concentration and storage steps. The involvement of the GA in this process is not clearly established. This pathway could be applied to the production of the fibrillar amyloid from ABPP in macrophage/microglial cells as suggested by Haass et al. (1992) and also for already mentioned plaque amyloid synthetic pathway in microglial cells (Wisniewski et al., 1986, 1990, 1991, 1992).

Acknowledgements. The authors thank Ms. M. Stoddard Marlow for copyediting the manuscript and Ms. J. Kay for secretarial assistance. MAAb 4G8 was the generous gift of Dr. K.S. Kim from IBR. The study was supported by funds from the New York State Office of Mental Retardation and Developmental Disabilities and a grant from Aventis Pharmaceuticals, Bridgewater, NJ (USA).

References

- Barka T. and Anderson P.J. (1962). Histochemical methods for acid phosphatase using hexazonium pararosanilin as coupler. *J. Histochem. Cytochem.* 10, 741-753.
- Cork L.C., Masters C., Beyreuther K. and Price D.L. (1990). Development of senile plaques. Relationships of neuronal abnormalities and amyloid deposits. *Am. J. Pathol.* 137, 1383-1392.
- Haass Ch., Koo E.H., Mellon A., Hung A.Y. and Selkoe D.J. (1992). Targeting of cell-surface β -amyloid precursor protein to lysosomes: alternative processing into amyloid-bearing fragments. *Nature* 357, 500-503.
- Hand A.R. and Oliver C. (1977). Cytochemical studies of GERL and its role in secretory granule formation in exocrine cells. *Histochem. J.* 9, 375-392.
- Kawarabayashi T., Shoji M., Sato M., Sasaki A., Ho I., Eckman C.B., Prada C.M., Younkin S.G., Kobayashi T., Tada N., Matsubara E., Harigaya Y., Kasai K. and Hirai S. (1996). Accumulation of β -amyloid fibrils in pancreas of transgenic mice. *Neurobiol. Aging* 17, 215-222.
- Kawarabayashi T., Igeta Y., Sato M., Sasaki A., Matsubara E., Kanai M., Tomidokoro Y., Ishiguro K., Okamoto K., Hirai S. and Shoji M. (1997). Lysosomal generation of amyloid β protein species in transgenic mice. *Brain Res.* 765, 343-348.
- Kim K.S., Miller D.L., Sapienza V.J., Chen C.M.J., Bai C., Grundke-Iqbal I., Currie J.R., Wisniewski H.M. (1988). Production and characterization of monoclonal antibodies reactive to synthetic cerebrovascular amyloid peptide. *Neurosci. Res. Commun.* 2, 121-130.
- Koning de E.J.P., Brand van den J.J.G., Mott V.L., Charge S.B.P., Hansen B.C., Bodkin N.L., Morris J.F. and Clark A. (1998). Macrophages and pancreatic islet amyloidosis. *Amyloid: Int. J. Exp. Clin. Invest.* 5, 247-254.
- Leskes A., Siekevitz P., Palade G.E. (1971). Differentiation of endoplasmic reticulum in hepatocytes. I. Glucose-6-phosphatase distribution in situ. *J. Cell Biol.* 49, 261-287.
- Manns E. (1968). Preservation of glucose 6-phosphatase activity in formaldehyde-fixed fresh frozen sections. *J. Histochem. Cytochem.* 16, 819-822.
- Novikoff A.B. (1963). Lysosomes in the physiology and pathology of cells. Contribution of staining methods. In: Ciba foundation symposium on lysosomes. de Reuck A.V.S. and Cameron M.P. (eds). Little, Brown, Boston. pp 36-77.
- Novikoff A.B. (1976). The endoplasmic reticulum: A cytochemist's view. *Proc. Natl. Acad. Sci. USA* 73, 2781-2787.
- Novikoff A.B. and Goldfischer S. (1961). Nucleoside diphosphatase activity in the Golgi apparatus and its usefulness for cytological studies. *Proc. Natl. Acad. Sci. USA* 47, 802-810.
- Novikoff A.B. and Essner E. (1962). Pathological changes in cytoplasmic organelles. *Fed. Proc.* 21, 1130-1142.
- Novikoff A.B. and Novikoff P.M. (1972). Cytochemical staining reactions for enzymes in cytoplasmic organelles. In: Biomembranes. Manson E.A. (ed). Plenum Press, New York. pp 33-39.
- Novikoff A.B., Mori M., Quintana N. and Yam A. (1977). Studies of the secretory process in the mammalian exocrine pancreas I. The condensing vacuoles. *J. Cell Biol.* 75, 48-165.
- Novikoff A.B., Quintana N. and Mori M. (1978). Studies on the secretory process in exocrine pancreas cells II. C57 black and beige mice. *J. Histochem. Cytochem.* 26, 83-93.
- Palade G. (1975). Intracellular aspects of the protein synthesis. *Science* 189, 347-358.
- Pappolla M.A., Omar R.A. and Vinters H.V. (1991). Image analysis microspectroscopy shows that neurons participate in the genesis of

Cell organelles and amyloid formation

- a subset of early primitive (diffuse) senile plaques. *Am. J. Pathol.* 39, 599-607.
- Powers J.M. (1986). Senile cerebral amyloid-evidence for a neuronal origin of the fibril protein. In: *Amyloidosis*. Glenner G.G., Osseman E.F., Benditt E.P., Calkins E., Cohen A.S. and Zucker-Franklin D. (eds). Plenum Press. New York. pp 743-749.
- Price L.P., Thinkaran G., Borchelt D.R. Martin L.J., Crain B.J., Sisodia S.S. and Troncoso J.C. (1998). Neuropathology of Alzheimer's disease and animal models. In: *Neuropathology of dementing disorders*. Arnold, London, pp 121-141.
- Probst A., Langui D., Ipsen S., Robakis N. and Ulrich J. (1991). Deposition of beta/A4 protein along neuronal plasma membranes in diffuse senile plaques *Acta Neuropathol.* 83, 21-29.
- Shoji M., Kawarabayashi T., Sato M., Sasaki A., Matsubara E., Igeta Y., Kanai M., Tomidokoro Y., Shizuka M., Ishiguro K., Harigaya Y., Okamoto K. and Hirai S. (1998). Accumulation of amyloid β protein in transgenic mice. *Neurobiol. Aging* 19, S59-S63.
- Vorbrodt A.W. and Wisniewski H.M. (1982). Plasmalemma-bound nucleoside diphosphatase as a cytochemical marker of central nervous system (CNS) mesodermal cells. *J. Histochem. Cytochem.* 30, 418-424.
- Wegiel J. and Wisniewski H.M. (1999). Projections of neurons in neuritic plaques formation. *Neurosci. News* 2, 34-39.
- Wegiel J., Wisniewski H.M., Muzylak M., Tarnawski M., Badmaev E., Nowakowski J., Wang K.C., Shoji M., Mondadori C. and Giovanni A. (2000). Fibrillar amyloid- β production, accumulation, and recycling in transgenic mice pancreatic acinar cells and macrophages: *Amyloid. Int. J. Exp. Clin. Invest.* 7, 95-104.
- Wisniewski H.M., Vorbrodt A.W., Moretz R.C., Lossinsky A.S. and Grundke-Iqbal I. (1982). Pathogenesis of neuritic (senile) and amyloid plaque formation. *Expt. Brain Res. (Suppl. 5)*, 3-9.
- Wisniewski H.M., Iqbal, K., Grundke-Iqbal I., Rubenstein R, Wen G.Y., Merz P.A., Kascsak R. and Kristensson K (1986). Amyloid in Alzheimer's disease and unconventional viral infections. *Neuropathology (Suppl. 3)*, 87-94.
- Wisniewski H.M., Vorbrodt A.W., Wegiel J., Morys J. and Lossinsky A.S. (1990) Ultrastructure of the cells forming amyloid fibers in Alzheimer Disease and Scrapie. *Amer. J. Med. Gen. (Suppl. 7)*, pp 287-297.
- Wisniewski H.M., Vorbrodt A.W. and Epstein M.H. (1991). Nucleoside diphosphatase (NDPase) activity associated with human β -protein amyloid fibers. *Acta Neuropathol.* 81, 366-370.
- Wisniewski H.M., Wegiel J., Wang KC. and Lach B. (1992). Ultrastructure studies of the cells forming amyloid in the cortical vessel wall in Alzheimer's disease. *Acta Neuropathol.* 84, 117-120.

Accepted June 6, 2001