Electron Microscopy of the Heart in a Case of Primary Cardiac Amyloidosis

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Although electron microscope studies of the ultrastructure of amyloid and its location in kidney and other organs (Cohen and Calkins, 1959; Heefner and Sorenson, 1962; Gueft and Ghidoni, 1963) have been carried out, there are no reports of the electron microscopy of amyloid in the heart. This communication describes the electron microscope findings in the heart from a case of primary amyloidosis.

MATERIAL AND METHOD

The patient was a male Pakistani who died at the age of 35 from renal and cardiac amyloidosis. The duration of the illness was short, the first symptoms appearing only 3 months before death. The initial manifestations of the disease were those of the nephrotic syndrome, but intractable congestive cardiac failure developed shortly afterwards and was considered at necropsy to be the cause of death.

Post-mortem examination was carried out 16 hours after death. Only the relevant necropsy findings will be described.

There was moderate oedema of both lower limbs. Bilateral serous pleural effusions were present and there was a 300 ml. pericardial effusion. The liver and spleen were both enlarged (2000 g. and 395 g., respectively). Both showed severe passive venous congestion. The heart was enlarged (weight 550 g.), with uniform enlargement of all chambers. The myocardium was firmer than normal and appeared waxy. The tongue was enlarged and showed indentations from the teeth around the periphery. The thyroid gland was uniformly enlarged and weighed 50 g. The kidneys were equally enlarged (combined weight 425 g.); the parenchyma was paler and firmer than normal and waxy in appearance.

For light microscopy thin slices of cardiac and other tissues were fixed in 10 per cent formol-saline and embedded in paraffin wax. Sections were stained with haematoxylin and eosin, haematoxylin and van Gieson, periodic acid-Schiff, Congo-red, and crystal violet.

For electron microscopy formalin-fixed cardiac tissue was cut into 1 mm.³ blocks, washed in distilled water for

48 hours, refixed in 1 per cent buffered osmium tetroxide for 1 hour, and embedded in araldite. Thin sections on carbon-coated grids were stained with lead citrate and examined in an AEI EM6B electron microscope at an accelerating voltage of 60 kV.

OBSERVATIONS

Light microscopy showed extensive deposits of amyloid in heart (Fig. 1), thyroid, kidney, and tongue. The cardiac deposits were present in the interstitium of both atria and ventricles.

Electron Microscopy. Although the cardiac tissue was 16 hours *post mortem* before formalin-fixation, some cytological detail was recognizable; the myofibrils were well preserved, and Z bands and even the thick and thin filaments were clearly seen. The cell membrane, however, was partly disrupted and the mitochondria were swollen and their cristae fragmented.

Amyloid deposits were present in relation to the basement membranes of cardiac muscle cells (Fig. 2a), around capillaries (Fig. 2b), and in the connective tissues (Fig. 2c). At low magnifications the deposits appeared granular but at higher magnifications they were seen to consist of fine fibrils. Some of the fibrils were disposed haphazardly, others were arranged in bundles (Fig. 2d). The fibrils varied in width from 140A–450A; those of greatest diameter appeared to be present in the bundles. In some areas there was a suggestion of regular beading along the fibrils.

In the connective tissue amyloid fibrils and collagen fibrils were both present. It was easy to distinguish the much larger mature collagen fibres with their characteristic periodicity (600A) from the more slender amyloid fibrils. The mature collagen fibrils showed no loss of periodicity.

Extensive deposits of amyloid were present between the muscle cells which were reduced in size. Large numbers of mitochondria were

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FIG. 1.—Myocardium showing extensive amyloid deposits apparently replacing muscle fibres and extending between existing fibres. (Crystal violet. $\times 350$.)

present, however, and the myofibrils were easily recognizable. A few cells contained deposits of lipofuscin. The remains of the cell membrane were seen in some areas but in other areas it had disappeared and was replaced by masses of amyloid which formed a thick mass around the cell. Amyloid deposition inside the myocardial cells was not seen.

Only occasional capillaries were recognized, and where extensive deposits of amyloid were present in the connective tissue there seemed to be a reduction in the number of capillaries. Masses of amyloid surrounded those which were seen and in some areas the capillary basement membrane appeared to be replaced by amyloid. Occasionally the appearances suggested that amyloid was in direct contact with the capillary lumen.

A few cells of the macrophage type were seen in the connective tissues but no intracellular amyloid was observed.

DISCUSSION

Amyloid deposits in human primary and secondary amyloidosis and in experimentally induced amyloidosis, which have been studied in the electron microscope, have all been seen to have a fine fibrillary structure. A similar fibrillary appearance was recognized in the deposits in the heart in this case.

The width of the individual fibrils in the cardiac deposits varied between 140A–450A. There was

also considerable variation (75A–300A) in the width of the fibrils in previous reports of the ultrastructure of amyloid (Cohen, Weiss, and Calkins, 1960; Boéré, Ruinen, and Scholten, 1965). Gueft and Ghidoni (1963) observed that some fibrils appeared to be composed of two filaments and that cross-striations appeared to be present. Shirahama and Cohen in 1965 demonstrated by electron microscopy of negatively stained amyloid that each fibril was composed of from 1–8 filaments, each of which was 75A in diameter. This provides an explanation for the variation in the width of the fibrils.

The amyloid in this case was situated in the intercellular space in close relation to the plasma membrane of the muscle cells and the basement membrane of the capillaries. In some areas the capillary basement membrane appeared to have disappeared and amyloid seemed to be in contact with the lumen of the vessel. Although this may have been an artefact or possibly the result of autolytic change, it has been observed that basement membrane is well preserved in necropsy material (Ashworth and Stembridge, 1964). Moreover other authors have described a similar appearance in fresh osmium-tetroxide fixed material (Cohen *et al.*, 1960).

In many areas mature collagen fibres were present in close relation to amyloid deposits. The collagen did not appear to be taking part in the formation of the deposit. Evidence in support of this is the fact that isolated amyloid fibrils remain unchanged after

FIG. 2a.—Oblique section of muscle cell showing preserved myofibrils (My) and intercalated disc (ID) surrounded by amyloid (A). The basement membrane (BM) can be recognized in places. The mitochondria (MT) are swollen and show fragmentation of the cristae. ($\times 3700$.)

FIG. 2b.—Capillary surrounded by amyloid (A). The nuclei of two pericytes are prominent (N). The basement membrane (arrow) can be seen at some areas. ($\times 2700$.)

FIG. 2c.—Collagen fibrils (C) with well-defined characteristic periodicity together with amyloid fibrils (A) in connective tissue. ($\times 33,000$.)

FIG. 2d.—Amyloid fibrils, partly arranged haphazardly, and partly arranged in bundles. ($\times 27,000$.)

treatment with collagenase and hyaluronidase (Cohen and Calkins, 1964). The number of capillaries in the intercellular zones appeared to be reduced, a factor that may have been important in the production of muscle cell atrophy. The intractable congestive cardiac failure which is the commonest manifestation of cardiac amyloidosis (Lindsay, 1946; Benson and Smith, 1956) may be the result of myocardial atrophy and interference with cellular nutrition. It may however be the

result of mechanical interference with diastolic expansion and filling of the heart due to the presence of large amounts of relatively inelastic amyloid material. This is supported by cardiac catheterization studies which in cardiac amyloidosis give results similar to constrictive pericarditis (Gunnar *et al.*, 1955).

Although cytoplasmic changes presumably occur in muscle cells in advanced amyloidosis, any such changes in this case were obscured by autolysis.

Clumping of nuclear chromatin, swelling of mitochondria, and disruption of the cell membrane are early autolytic changes (Ashworth and Stembridge, 1964) and these were the only striking cellular changes seen in the myocardial cells. Although fresh osmium-fixed tissue is essential for cytological detail, certain components of connective tissue are well preserved in necropsy material (Lannigan and Zaki, 1965; Lehner, Nunn, and Pearse, 1966), and useful information may be obtained by electron microscopy of such material.

SUMMARY

The electron microscope appearances of post-mortem cardiac tissue from a case of primary cardiac amyloidosis are described. The mechanisms which may be responsible for congestive cardiac failure in myocardial amyloidosis are briefly discussed.

REFERENCES

- Ashworth, C. T., and Stembridge, V. A. (1964). Utility of formalin fixed surgical and autopsy specimens for electron microscopy. *Amer. J. clin. Path.*, **42**, 466.
- Benson, R., and Smith, J. F. (1956). Cardiac amyloidosis. *Brit. Heart J.*, **18**, 529.
- Boeré, N., Ruinen, L., and Scholten, J. H. (1965). Electron microscopic studies on the fibrillar component of human splenic amyloid. *J. Lab. clin. Med.*, **66**, 943.
- Cohen, A. S., and Calkins, E. (1959). Electron microscopic observations on a fibrous component in amyloid of diverse origins. *Nature (Lond.)*, **183**, 1202.
- , and — (1964). The isolation of amyloid fibrils and a study of the effect of collagenase and hyaluronidase. *J. Cell Biol.*, **21**, 481.
- , Weiss, L., and Calkins, E. (1960). Electron microscopic observations of the spleen during the induction of experimental amyloidosis in the rabbit. *Amer. J. Path.*, **37**, 413.
- Gueft, B., and Ghidoni, J. J. (1963). The site of formation and ultra-structure of amyloid. *Amer. J. Path.*, **43**, 837.
- Gunnar, R. M., Dillon, R. F., Wallyn, R. J., and Elisberg, E. I. (1955). The physiologic and clinical similarity between primary amyloid of the heart and constrictive pericarditis. *Circulation*, **12**, 827.
- Heefner, W. A., and Sorenson, G. D. (1962). Experimental amyloidosis. Light and electron microscope observations of spleen and lymph nodes. *Lab. Invest.*, **11**, 585.
- Lannigan, R., and Zaki, S. (1965). Electron microscope appearances of a fibrin-staining component in an Aschoff nodule from acute rheumatic fever. *Nature (Lond.)*, **206**, 106.
- Lehner, T., Nunn, R. E., and Pearse, A. G. E. (1966). Electron microscopy of paraffin-embedded material in amyloidosis. *J. Path. Bact.*, **91**, 297.
- Lindsay, S. (1946). The heart in primary systemic amyloidosis. *Amer. Heart J.*, **32**, 419.
- Shirahama, T., and Cohen, A. S. (1965). Structure of amyloid fibrils after negative staining and high-resolution electron microscopy. *Nature (Lond.)*, **206**, 737.