Electron microscopical study of myocardial biopsy material in congenital heart block

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Myocardial tissue from the left ventricle of two children with congenital atrioventricular block and recurrent Adams-Stokes attacks was obtained during the procedure of epicardial pacemaker implantation and studied with the electron microscope. One patient had a familial cardiomyopathy, the other had no clinical abnormality apart from the conduction defect. Proliferation of mitochondria was common in both cases. Focal interstitial fibrosis and pronounced proliferation of transverse tubules were found in the myocardial cells of the patient with familial cardiomyopathy. Deep invaginations of the sarcolemma with formation of intracytoplasmic channels were present in the second case. The possibility that the numerous transverse tubules could serve as additional pathways for impulse conduction is discussed.

Congenital complete atrioventricular block is usually associated with congenital cardiac malformations, but also occurs in patients without any apparent disease. This conduction disturbance has been described in relation to its clinical (Gazes et al., 1965; Kariv et al., 1971; Corne and Mathewson, 1972), haemodynamic (Scarpelli and Rudolph, 1964; Thilenius et al., 1972), and histological aspects (Lev et al., 1971; Carter, Blieden and Edwards, 1974), but the electron microscopy of the heart in congenital atrioventricular block in children has not been studied. Previous studies on the myocardial ultrastructure have been on adult patients with idiopathic cardiomyopathy (Hibbs et al., 1965; McCallister and Brown, 1967; Van Noorden, Olsen, and Pearse, 1971; Ferrans, Morrow, and Roberts, 1972; Bulloch et al., 1972), without any association with congenital heart block.

This paper reports on the ultrastructural findings in the myocardium of two children with congenital atrioventricular block. One case had a familial cardiomyopathy, the other had no clinical evidence of cardiac abnormality, apart from the conduction defect. Because of recurrent Adams-Stokes attacks, the implantation of a permanent pacemaker became necessary in both patients. During the epicardial implantation myocardial biopsies were obtained and these were examined by electron microscopy.

Case reports

Case I

A 13-year-old boy of Arabic origin. The parents were unrelated and three brothers were alive and healthy. Six other brothers died of unknown causes between 2 and 3 years of age.

The patient was admitted to hospital because of recurrent Adams-Stokes attacks during the previous year. He complained also of dyspnoea on effort. His general appearance was healthy for his age. The pulse rate was 36 a minute and regular, the blood pressure 105/55 mmHg (14.0/7.3 kPa). His liver was enlarged 4 fingers below the costal margin and not tender. Auscultation revealed a first heart sound of varying intensity, the presence of a third heart sound, and a grade 2/6 systolic ejection murmur at the left sternal border.

The electrocardiogram showed complete atrioventricular block and idioventricular escape rhythm complexes with the pattern of complete left bundle-branch block (Fig. 1). The atrial rate was 120 a minute and the ventricular rate 36 a minute. Radiographic examination of the chest revealed an enlarged heart, caused by enlargement of both ventricles, with normal lung fields. Routine laboratory examinations were all within normal limits. Twenty-four hours after the insertion of a temporary pacemaker, sinus rhythm reappeared with a normal PR interval. However, the QRS complexes remained unchanged. The electrocardiogram showed evidence of left atrial hypertrophy (Fig. 1).

On cardiac catheterization no shunt was demonstrated. There was no pressure gradient across the aortic valve. The end-diastolic pressure in both ventricles was
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FIG. 1 Electrocardiogram: A) Case 1 in sinus rhythm. B) Case 1 with complete atrioventricular block. C) Case 2 with complete atrioventricular block.

elevated. Right ventricle systolic pressure: 35 mmHg (4.7 kPa), end-diastolic pressure: 9 mmHg (1.2 kPa). Left ventricle systolic pressure: 110 mmHg (14.6 kPa), end-diastolic pressure: 35 mmHg (4.7 kPa). The mean pulmonary wedge pressure was 17 mmHg (2.3 kPa). The diagnosis of familial non-obstructive cardiomyopathy with transient recurrent complete atrioventricular block was made and a permanent demand pacemaker was implanted using epicardial electrodes. The patient made an uneventful recovery and is well and fully active now, two years after the implantation.

Case 2

A 14-year-old boy of Arabic origin. The family history was non-contributory. He was known to have had complete atrioventricular block since the age of 7. He was admitted to our hospital because of an Adams-Stokes attack. On examination he appeared normal for his age. The blood-pressure was 150/80 mmHg (20.0/10.6 kPa) and the pulse rate 40 a minute. There was no evidence of congestive heart failure. On auscultation the first heart sound was of varying intensity and a third sound was noted. A grade 3/6 ejection systolic murmur, maximal at the left sternal border, radiating to the base of the heart, was heard. The electrocardiogram showed complete atrioventricular block (Fig. 1) with an idioventricular escape rhythm showing the pattern of complete left bundle-branch block. Radiographic examination of the chest revealed an enlarged heart with enlargement of both ventricles. Routine laboratory investigations were all within normal limits.

On admission a temporary pacemaker was inserted. Right heart catheterization while the patient was paced revealed no shunts and normal right atrial, right ventricular, pulmonary artery, and wedge pressures. A demand epicardial pacemaker was implanted. The patient made an uneventful recovery and remains well now, 8 months after the pacemaker implantation.

Methods

Myocardial specimens were obtained from the subepicardial area of the left ventricle during the procedure of epicardial pacemaker implantation. The sample was divided into two parts: the larger part was processed in the usual manner for light microscopy. The second part was immediately cut into small pieces and fixed for one hour with cold 3 per cent glutaraldehyde, post fixed with 2 per cent osmium tetroxide, dehydrated by alcohols, and embedded in Epon. Sections 1 μ thick were stained with toluidine blue for light microscopy. Thin sections were stained with uranyl acetate and lead citrate and examined with a Zeiss 9 S electron microscope.

Results

Case 1

Light microscopy The muscle fibres appeared to be in a disorderly arrangement. The nuclei varied much in size and shape. There was no evidence of connective tissue by routine and specific stains.
Electron microscopy The myofibres appeared to be in a contracted state. Disorganization of the sarcomere pattern and focal disruption of myofilaments was a constant finding. The disrupted areas were occupied by clusters of mitochondria of which the great majority appeared undamaged, but occasionally an enlarged mitochondrion with disrupted cristae was encountered. In many areas the myofilaments were coursing in diverging directions. Disorientation of myofilaments was often noted in the vicinity of the intercalated disc. The transverse tubules (tubules of the T-system) were numerous. They were either U-shaped and facing the Z-line, or oriented in a distorted manner parallel to the myofilaments (Fig. 2). Dense material similar to that of the basement membrane was lining the inner side of the transverse tubules (Fig. 2). Vesicles and narrow tubules of sarcoplasmic reticulum were related to the transverse tubules (Fig. 2). Irregular shaped foci of electron dense material were sometimes associated with the intercalated disc or with the transverse tubules (Fig. 3). Glycogen pads were commonly situated in the proximity of the nucleus. In many places a large amount of collagen fibrils in the interstitial space between myocardial cells was seen to separate the cells from each other (Fig. 4). Other regions were free of collagen tissue. Another finding was an increase in the number of non-myelinated nerve fibres. The axons were filled with granular and agranular vesicles (Fig. 5). True myoneural junctions (nerve-muscle close contact) have not been depicted.

Case 2

Light microscopy The muscle fibres were conspicuously hypertrophic with variations in size and shape, and separated by vacuolar connective tissue. In a few fibres empty spaces of different size and shape were present; usually they were single and large. The spaces were sometimes related to the nucleus which was pushed to the periphery of the cell forming a signet ring kind of cell.

FIG. 2 Electron micrograph from Case 1. Longitudinal section of a contracted myocardial cell showing the sarcomere pattern slightly disrupted by a small cluster of mitochondria (M), and dispersed glycogen granules. U-shaped or distorted transverse tubules (arrows) have dense material applied on their inner side. Z = Z-line. (× 19 000.)
FIG. 3 Case I. An intercalated disc on the left side of the picture is associated with small masses of dense material. Foci of electron dense material are seen in the centre of the picture, in the vicinity of the sarcoplasmic tubules. Loss of cristae in a mitochondrion is seen in the upper right corner. ($\times$ 19,000.)

Electron microscopy The cardiac muscle cells showed irregular cellular contraction. Deep indentations of the sarcolemma were frequently seen. In many places the invaginated sarcolemma extended deep into the cell and created irregular intracytoplasmic slits and spaces which were lined by the basal lamina of the sarcolemmal membrane (Fig. 6). The nuclei showed unusual deep circumvolutions and in this way cytoplasmic organelles were caught between the indentations (Fig. 7). The invaginated regions probably correspond to the perinuclear empty spaces seen by light microscopy. Areas of condensed mitochondria were common (Fig. 8). The cristae mitochondriales were often disrupted, leaving round-shaped spaces inside the mitochondria. The loss of cristae was variable, to the degree of transforming the mitochondrion into a huge, double membrane bounded vesicle (Fig. 9). Some vesicles were up to 5 μ in diameter and totally empty. The only resemblance to the mitochondria was because of their double wall. Dense bodies and lysosomes containing lipofuscin occurred mostly in areas of accumulated mitochondria. There were few tubules of sarcoplasmic reticulum in regions with damaged sarcosomes, but in other places the tubules were well represented. Focal degeneration of myofilaments was evident in the vicinity of the accumulated mitochondria. No changes were apparent in the morphology of the intercalated disc.

Discussion In both patients the proliferation of mitochondria (mitochondriosis) was remarkable. Mitochondriosis has been reported in adults with obstructive cardiomyopathy (Pearse, 1964; Van Noorden, Olsen, and Pearse, 1971), in alcoholic cardiomyopathy (Alexander, 1966), and in a lesser degree in cases of idiopathic cardiomyopathy (Ferrans et al., 1972; Bulloch et al., 1972). The massive loss of cristae mitochondriales in our second case, to the extent of creating cytoplasmic giant vesicles, seems to be a non-specific feature. Similar appearances were experimentally obtained in dogs given emetine hydrochloride (Pearce, Bulloch, and Murphy, 1971), and have been described as 'moth-eaten'
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FIG. 4 Case I. A large amount of collagen fibrils form a wide band in the interstitial space between cells, and thus completely separate two myocardial cells from each other. The collagen fibrils are transversely sectioned, with only a few longitudinally oriented fibrils among them. The muscle cells are in a contracted state. (× 14 700.)

mitochondria in hearts from alcoholics (Alexander, 1966). Large, double-membrane enclosed vesicles were observed in cases of idiopathic and alcoholic cardiomyopathy (Burch and DePasquale, 1969; Bulloch et al., 1972), but their relation to mitochondria was not emphasized. We excluded the possibility of artefactual changes in the mitochondria on the ground of normal distribution of the nuclear chromatin and good preservation of different cytoplasmic components.

Histological studies on necropsy material in congenital heart block demonstrated aberrant fibrous tissue at the level of the atrioventricular node or at the atrioventricular bundle, which was thought to block or to interrupt the conduction pathways (Lev et al., 1971; Carter et al., 1974). We do not know, in our cases, what the morphological changes in the atrioventricular node are, if any, since this study was done on biopsy material from the surface of the left ventricle. In the case of familial cardiomyopathy a relatively large amount of collagen fibrils was found in the interstitial space between myocardial cells. The collagen tissue had a patchy distribution and may have produced focal disturbances in the spread of the wave of membrane depolarization from one cell to another. Interstitial collagen tissue was also revealed ultrastructurally by Pearse (1964) and by Ferrans et al. (1972) in
FIG. 5 (legend opposite)

FIG. 6 (legend opposite)
FIG. 7 Case 2. An enlarged nucleus shows multiple deep circumvolutions. A cytoplasmic mass with condensed mitochondria was caught in the indentations of the nucleus (arrow). Clusters of mitochondria (M) are also seen around the nucleus. Note the well-preserved cristae of the mitochondria. (× 9800.)

FIG. 5 Case 1. A non-myelinated nerve fibre is seen in the vicinity of the sarcolemma of a cardiac muscle cell. The axonal granular vesicles are well represented. A few agranular vesicles are also seen. (×19500.)

FIG. 6 Case 2. Obliquely cut section of a contracted muscle cell. The sarcolemma on the right of the picture is deeply indented, thus forming intracytoplasmic channels. A cluster of mitochondria is seen in the left upper corner. (× 9800.)
**FIG. 8** Case 2. A conglomeration of mitochondria occupies most of the field. The cristae mitochondriales are well represented except for a few places where small empty spaces are present (arrow). The myofilaments are in a state of myolysis. L = lipid droplet (× 19 000.)

**FIG. 9** Case 2. Enlarged picture that illustrates the pronounced loss of cristae mitochondriales. One mitochondrion has been transformed into an empty, double membrane bounded vesicle, with only two cristae left inside (arrow). L = lipid droplet. (× 56 000.)
obstructive cardiomyopathy (idiopathic hypertrophic subaortic stenosis).

The role played by the transverse tubules and by the other tubules of the sarcoplasmic reticulum in the transmission of intercellular impulses is well known (Porter and Palade, 1957). In this connexion, it is tempting to speculate that the numerous transverse tubules, noted in our case of familial cardiomyopathy, could serve as additional pathways for impulse conduction.

In our second case, the multiple deep sarcolemmal infoldings that obviously increased the cell membrane surface, and the newly formed cytoplasmic slits, could be a compensatory mechanism which supplies the muscle cell with more membrane surface for transport of ions important in the electrical phenomena of myocardial excitation.

The link between transverse tubules and sarcolemma is well established by the work of many investigators demonstrating that the transverse tubules consist of deep invaginations of the sarcolemmal surface of the cell (Nelson and Benson, 1963; Franzini-Armstrong and Porter, 1964; Simpson, 1965).

Infoldings of the cell membrane are commonly associated with cardiac hypertrophy and were ultrastructurally described in the human ventricle (Dowlatabadi and Hunt, 1969), but in their material the sarcolemmal invaginations did not extend so very deep inside the cell and did not form intracytoplasmic channels.

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