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Ultrastructural effects induced by global ischaemia on the AV node compared with the working myocardium

A qualitative and morphometric investigation on the canine heart*

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Summary. The cardiac conduction system is considered to be particularly resistant to ischaemia. Nevertheless, following open heart surgery with short periods of ischaemia disturbances in AV conduction or ventricular arrhythmia have been reported. We compared the ultrastructure of AV node and working myocardium following 30 min global ischaemia at 25° C, during pure ischaemia and with HTK cardioplegia qualitatively and morphometrically. After 30 min of pure ischaemia, interstitial and intracellular oedema together with considerable changes in organelles in AV nodes predominate over mainly cellular oedema in working myocardium. Sometimes irregular overcontractions of sarcomeres occur in the AV node, though very seldom in working myocardium. In pure ischaemia, mitochondrial swelling is comparable in both types of tissue. Following HTK cardioplegia and 30 min ischaemia, cellular oedema and mitochondrial swelling are significantly reduced in AV nodal cells and working myocardium, but remain more extensive in the AV nodes. Irregularities in the contractile state of sarcomeres are not observed. The extent of the ultrastructural alterations corresponds to the degree of metabolic change in the working myocardium. Thus, despite considerable differences during pure ischaemia and HTK cardioplegia, ultrastructurally the AV nodal cells do not display a greater resistance to ischaemia than working myocardium.

Key words: AV nodal cells – Working myocardium – Cardiac arrest and global ischaemia – HTK cardioplegia – Qualitative and quantitative ultrastructure

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Dedicated to Prof. Dr. med. Drs. h.c. Wilhelm Doerr, on the occasion of his 75th birthday

Introduction

The cardiac conduction system (CCS) is considered to be particularly resistant to ischaemia on the basis of structural, histochemical, metabolic and functional properties (Schiebler et al. 1956; Coffman et al. 1960; Kübler et al. 1985; for reviews see: Pick 1924; Doerr and Schiebler 1963; Schiebler and Doerr 1963; Kawamura and James 1971; Schneider 1981; Canale et al. 1986; Meijler and Janse 1988). There are few publications giving quantitative or morphometric data on the AV node in the absence of ischaemic stress (Marino 1979; Olivetti et al. 1979). Reports on fine structural changes in parts of the CCS indicating particular sensitivity of this system are relatively rare (Doerr 1957, 1959, 1972; Lierse et al. 1974).

The assumption of a higher tolerance to ischaemia was supported by clinical changes following myocardial infarction, where the working myocardium is more often and more seriously affected than the CCS. Certain areas of the CCS are, however, differently supplied by collaterals in different species, such as the region of the AV node (Doerr 1957; Doerr and Schiebler 1963; Schiebler and Doerr 1963; James and Sherf 1968; Schneider 1981; Canale et al. 1986).

In global ischaemia, characterised by a total cessation of the coronary circulation, pathological mechanisms are more uniform than for regional ischaemia (Bretschneider 1964; Bretschneider et al. 1975; Jennings and Reimer 1981; Kübler et al. 1985; Gebhard et al. 1989). The supply of oxygen and substrates as well as the removal of carbon dioxide and metabolites are halted completely.

In open heart surgery, cardiac arrest lasting two to three hours can be tolerated through the application of so-called myocardial protective methods. Nevertheless, references to a variety of arrhythmias which complicate post-ischaemic recovery after only short periods of ischaemia are found in the literature (Adappa et al. 1978; Smith et al. 1983; Flameng et al. 1984; Scheld 1984; Niederberger von Wolfenschiessen 1987; Walter et al. 1988).

Following pure global ischaemia, produced by single or intermittent cross clamping of the aorta with short periods of ischaemia, atrioventricular (AV) and fascicular cardiac conduction disturbances occur (Coffman et al. 1960; Bagdonas et al. 1961; Flameng et al. 1984; Kübler et al. 1985) together with ventricular extrasystole, tachycardia or even ventricular fibrillation (Coffman et al. 1960; Bagdonas et al. 1961; Adappa et al. 1978; Flameng et al. 1984) depending on duration of ischaemia and temperature. Use of cardioplegic solutions often results in a temporary AV block, although for certain methods, less reversible fascicular blocks and ventricular arrhythmias are reported (Flameng et al. 1984; Scheld 1984; Niederberger von Wolfenschiessen 1987). Detailed investigations using His-bundle-electrocardiography showed AV conduction disturbances specifically in the AV node after 30 min of global ischaemia, in spite of the use of myocardial protective methods (Smith et al. 1983), confirming the findings for pure ischaemia (Bagdonas et al. 1961).

More recent ultrastructural reports cast doubt on the idea of a generally higher tolerance of the CCS to ischaemia when compared with the working myocardium (Lierse et al. 1974; Armiger and Knell 1986; Richter et al. 1986, 1989; Schnabel et al. 1988; Clavien et al. 1989b; cf. with DiBona and Powell 1980; Jennings and Reimer 1981; Schnabel et al. 1987). There are, however, a few reports in the literature on systematic electron microscopical investigations on ischaemia-dependent changes of the AV node in dogs (Armiger and Knell 1986; Clavien et al. 1989b; Richter et al. 1989) which are comparable to the human being with respect to fine structure in the regions under investigation (James and Sherf 1968; Canale et al. 1986). Quantitative reports on this subject are less common (Clavien 1989; Clavien et al. 1989a; Richter et al. 1989). Doerr (1972) stated, "The morphometrical analysis of the elements of the CCS is one of the most important problems of our time."

We therefore examined the ultrastructure of the AV nodal cells and of the working myocardium of the left ventricle following 30 min of global ischaemia at 25° C in the dog heart. The results with and without myocardial protection (HTK cardioplegia and pure ischaemia respectively) were compared with reference values obtained without ischaemic stress.

Materials and methods

In combined neuroleptic analgesia, the hearts of a total of 26 dogs were arrested and thereafter subjected to a period of global ischaemia (Coffman et al. 1960; Bretschneider et al. 1975, 1983; Flameng et al. 1984; Scheld 1984; Schnabel et al. 1987; Walter et al. 1988; Gebhard et al. 1989). Pure ischaemia was induced by cross clamping of the aorta after inflow occlusion after topical cooling with Tutofusin solution (composition, see below) at 4° C. The empty beating hearts were vented by incision at the tip of the left ventricle. Mechanical arrest was achieved by ventricular fibrillation. Visible fibrillation, response to mechanical stimulation and electrical activity persist for a few minutes or longer depending on the temperature (Bretschneider 1964).

For HTK cardioplegia, coronary perfusion with HTK solution at 8° C (composition, see below) according to the method of Bretschneider, leads to mechanical and subsequent electrical cardiac arrest within 10–15 s by reduction of the extracellular sodium and calcium concentrations to sarcoplasmic values (Gebhard et al. 1984). In order to equilibrate the entire extracellular space, the perfusion was continued for 11 min. The left ventricle was vented and the cardiac atria opened to release the solution.

Tutofusin (Pfrimmer & Co., Erlangen, FRG) is composed of 140 mmol NaCl, 5 mmol KCl, 2.5 mmol $CaCl_2$, 1.5 mmol $MgCl_2$ per liter; the cardioplegic solution HTK according to Bretschneider (Dr. F. Koehler Chemie GmbH, Alsbach, FRG) of 15 mmol NaCl, 9 mmol KCl, 4 mmol $MgCl_2$, 180 mmol histidine, 18 mmol histidine-HCl, 2 mmol tryptophan, 1 mmol K-α-ketoglutarate and 30 mmol mannitol per liter.

Immediately following the onset of pure ischaemia or after completion of HTK perfusion, the first samples for fine structural and biochemical analyses were taken from the tip of the left ventricle. Thereafter, the hearts were removed and incubated at 25° C in Tutofusin or HTK solution. The interstitial pH was measured continually in the ventricular septum by means of glass implant electrodes. Further samples from the free wall of the left ventricle were taken at defined time points in the course of ischaemia. The tissue concentrations of creatine phosphate (CP), adenosine triphosphate (ATP), glycogen or lactate were measured enzymatically (Gebhard et al. 1984; Schnabel et al. 1987).

Before the AV node was dissected, the ventricles (below the level of the valves) were removed and incubated. The AV nodes themselves were dissected according to the method of Davies (cited from Schneider 1981) (Schünemann et al. 1988; Clavien 1989). Up to the final fixation of the small samples

of the AV nodes for electron microscopy, the period of ischaemia is about 30 min at approx. 21° C (Clavien et al. 1989b; Richter et al. 1989). The samples were fixed by immersion in a fixation solution composed of 1.5% glutaraldehyde, 1.5% paraformaldehyde in 0.1 M sodium cacodylate buffer. Perfusion fixation immediately after HTK cardioplegia was carried out with the same fixative as recently published (Richter et al. 1984; Schnabel et al. 1985; Schmiedl et al. 1989a). The samples were further processed with a HISTOMAT (Biomed, Theres, FRG) before being embedded in araldite as already described in detail (Schnabel et al. 1988; Schmiedl et al. 1989b).

Three samples from the left ventricle for each animal and time point were taken by random selection from a total number of 10 to 15 blocks for the electron microscopic investigations. From the samples of the AV nodes (Clavien 1989; Richter et al. 1989), semithin and ultrathin sections were cut from three levels of the superficial part of the compact node (Canale et al. 1986; Sandusky et al. 1986; Schünemann et al. 1988). For each sample, 5 ultrathin sections were cut following the method of "random sectioning" (Weibel 1979). From each, one section was investigated qualitatively, quantitatively and morphometrically with an electron microscope (EM 10, Zeiss, Oberkochen, FRG). The quantitative and morphometrical evaluations were carried out online with a TV camera. Following "systematic random sampling" (Weibel 1979), 50 fields per section were analysed for morphometry (Schmiedl et al. 1989b).

Based on the point counting system of Weibel (1979), at a final magnification at 45500×, using a 72 lattice test system with test lines, the volume densities (V_V) of the myofibrils (V_{VMf}), the sarcoplasm (V_{VSp}), the mitochondria (V_{VMi}) and the cell nuclei (V_{VCn}) were measured using the cells as reference space. From each of the three edge points of the 24 angles of our lattice test system superimposed on the working myocardium, those points which fell on the structures of interest were counted (Clavien 1989; Schmiedl et al. 1989b). Furthermore the surface to volume ratio of mitochondria ($S_V ratio_{Mi}$) was used as a parameter for form and size of mitochondria independent of the reference space (Mall et al. 1986; Schmiedl et al. 1989b). The $S_V ratio_{Mi}$ is calculated by relating the intersections of the test lines with the outer membrane of the mitochondria to the test points superimposed on the mitochondria, using the method of Weibel (1979) (Mall et al. 1986; Schmiedl et al. 1989b).

All results are given as mean values ± SD unless indicated otherwise. Significant differences have been noted for P values of 0.05 or less using the Wilcoxon-Mann-Whitney (U) test, for unpaired samples or the Wilcoxon matched pairs signed rank test.

Results

In the following, the qualitative characteristics of AV nodes and working myocardium are depicted as they appear in an optimal preservation without ischaemia following HTK cardioplegia and immediate perfusion fixation. Thereafter, we report the results for pure ischaemia and HTK cardioplegia, at the onset of ischaemia and after 30 min of ischaemia, following immersion fixation.

The electron microscopical picture of the superficial part of the compact AV node following perfusion fixation is very complicated (Fig. 1). In the very narrow interstitial space between groups of

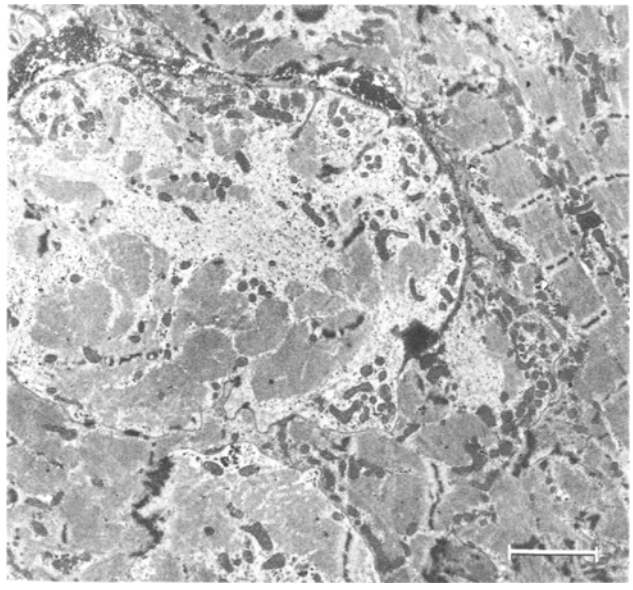

Fig. 1. Ultrastructure of the AV node, immediately after HTK perfusion and subsequent perfusion fixation (× 5600; scale bar: 2 μm)

nodal cells, we find capillaries, nerves which are largely unmyelinated showing multiple axons, fibrocytes and variable packing of bundles of connective tissue, predominantly collagen. The spindle shaped branched nodal cells are woven together. Their average thickness is between 3 and 10 μm. The cellular junctions of the nodal cells consist mainly of undifferentiated areas with two membranes often running parallel over long distances (Figs. 1, 2B). Between these are desmosomes. Very seldom are gap junctions seen.

The nodal cells possess significantly more so-called free sarcoplasm (Fig. 1), fewer myofibrils (Fig. 2B) and smaller mitochondria (Figs. 1, 2B) than the working myocardium. In the free sarcoplasm there are numerous granules, composed mainly of glycogen and ribosomes, also a few filamentary structures and parts of the sarcoplasmic reticulum. A transverse tubulus system (T-system) is absent. The myofibrils are arranged three-dimensionally within the nodal cells. Thus, they are rarely sectioned longitudinally over long distances (Figs. 1, 2B). Normally, the chromatin of the cell nucleus is finely distributed without much margination. The Golgi-apparatus is to be found with the rough endoplasmic reticulum in a perinuclear distribution. Between the compartments of the contractile system we find widely spread, very slender and sometimes branched mitochondria. These are sometimes piled up at the periphery of a glycogen-rich area (Figs. 1, 2B).

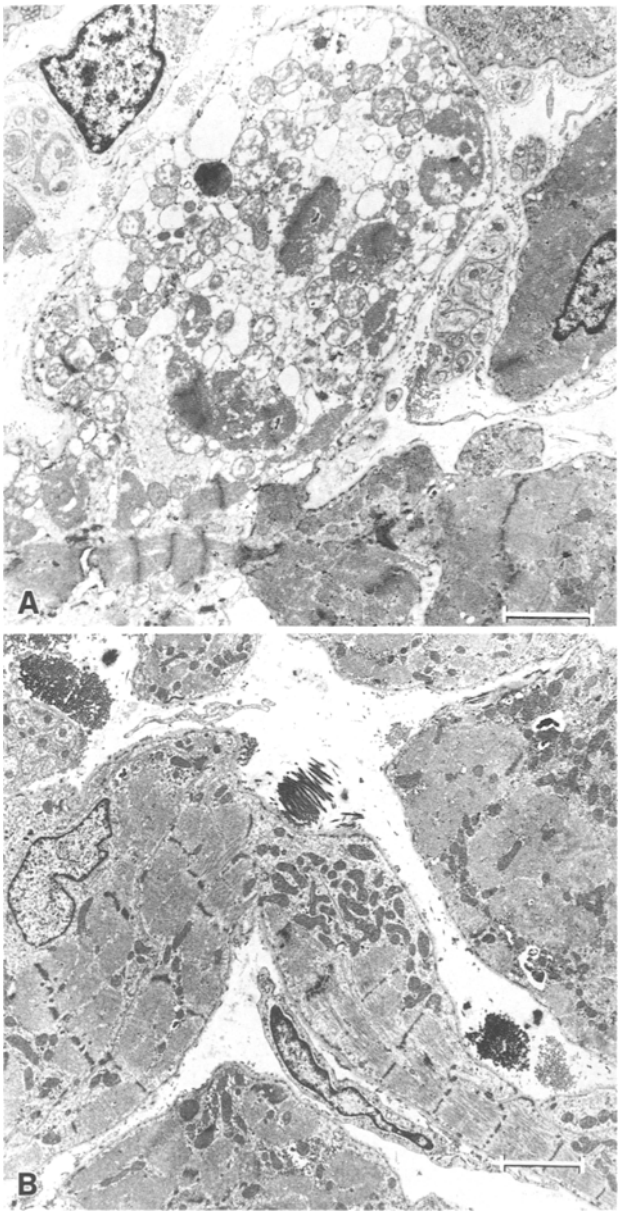

Fig. 2. Ultrastructure of AV nodal cells, 30 min of global ischaemia (≈21° C). **A** pure ischaemia; **B** HTK cardioplegia (immersion fixation, × 5600; scale bar: 2 μm)

After 30 min of pure ischaemia (≈21° C), the fine structure of the AV node appears very non-homogeneous (Fig. 2A). The interstitial space is enlarged to a differing extent; even neighbouring sections of the nodal cells of one group present a picture which is very rich in contrasts. Some show clear expansion due to oedema and are therefore very light in colour, others are hardly swollen at all and look rather condensed. Changes in the cellular junctions such as dehiscence of the undifferentiated regions or formation of vacuoles are only seen in connection with extensive cell oedema or irregular overcontraction of the sarcomeres (Fig. 2A). The sarcoplasm of the oedematous nodal cells appears to be lacking in structure in some of the areas (Figs. 2A, cf. 5A). In others, it is composed of clearly swollen mitochondria and also a significant number of vacuoles which probably mainly reflect dilated sarcoplasmic reticulum. The bundles of myofibrils in these cells appear separated (Figs. 2A, cf. 5B). The chromatin of the cell nucleus already shows an increased margination and clumping. The mitochondria are particularly severely swollen in the oedematous nodal cells (Figs. 2A, cf. 5C, 5D). They show a lightening of the matrix, loss of matrix, fragmentation of the cristae and even distended areas with cristolysis. This damage is less pronounced in nodal cells which are less swollen, but still present in such cells to varying degree.

After HTK perfusion and 30 min of ischaemia (≈21° C), the superficial AV nodes are much more uniform in appearance (Fig. 2B). The interstitial space appears homogeneous and moderately wide, also the connecting tissue fibres are packed closer together. Since the greater part of the nodal cells shows no or only slight cellular oedema, the overall cell picture is quite uniform (Figs. 2B, cf. 5A). Changes at the cellular junctions are not evident. Longitudinal sarcomeres show relatively even, narrow I-bands (Fig. 2B). Overcontractions or contraction bands are nowhere to be seen. In the predominantly longitudinally sectioned nodal cells, the free sarcoplasm between and beside the more relaxed part of the contractile system is much more clearly seen (Fig. 2B, cf. 5A, 5B). It is composed of numerous granula, finely distributed. Vacuoles or dilated sarcoplasmic reticulum are very rare. The nuclei show an even distribution of chromatin without clumping. Only in the case of sections not cut centrally do we see a somewhat stronger margination (Fig. 2B). The mitochondrial matrix is usually dark, the cristae appear intact and relatively closely packed. Matrix granula are, however, extremely rare. A slight mitochondrial swelling is recognizable in the sometimes bizarrely shaped organelles (Figs. 2B, cf. 5C, 5D).

Compared with the different parts of the CCS, the working myocardium shows a higher degree of structural preservation. This is true for the organisation of myocytes in bundles, as well as for the pattern of the cellular substructures. In the intramural working myocardium, at a distance of 400 ± 100 μm from the endocardium, the structure of the myocyte bundles appears to be very compact (Fig. 3). Fibres of connective tissue, visible with

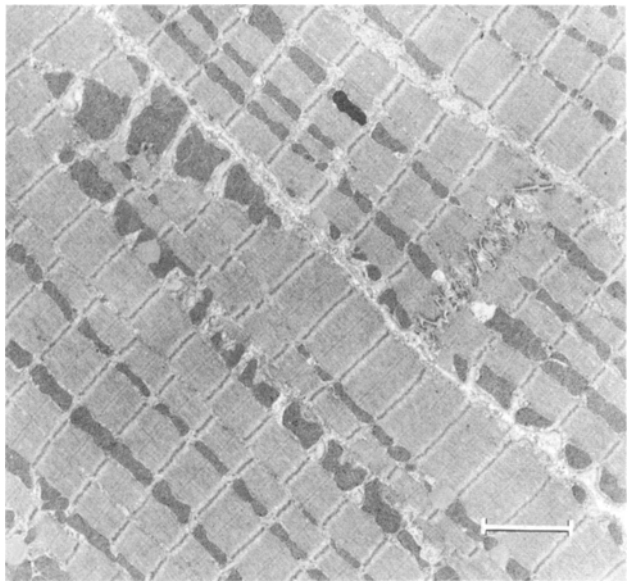

Fig. 3. Ultrastructure of left ventricular working myocardium immediately after HTK perfusion and subsequent perfusion fixation (× 5600; scale bar: 2 µm)

the light microscope, surround the myocyte bundles and, following immersion fixation, are mostly to be found in tissue clefts separating the bundles. In the working myocardium, the heart muscle cells are connected by highly digitated intercalated discs with numerous desmosomes, the connections being mainly end-to-end (Fig. 3).

In the myocytes of the working myocardium, the myofibrils which are always longitudinally oriented are separated only by narrow strips of free sarcoplasm and regularly ordered large elongated mitochondria (Figs. 3, 4C, 4D, cf. 5A, 5B). In the free sarcoplasm, we find numerous glycogen granules, and occasionally parts of the sarcoplasmic reticulum. Sections of the sarcolemmal invaginations which form the T-system are recognizable in longitudinally cut myocytes (Figs. 3, 4A–4D). The cell nuclei also show a fine distribution of chromatin in the working myocardium which, however, becomes more dense in the region of the nuclear membrane than it does in the CCS. The oval mitochondria are evidently larger than in the cells of the CCS and have numerous densely packed cristae (Figs. 3, 4C, 4D, cf. 5C, 5D). In the dark finely grained matrix, abundant normal matrix granules are to be found.

At the onset of pure ischaemia, immediately after aortic cross clamping, the structure of the working myocardium appears largely intact. The interstitial space is relatively wide, dehiscences of the intercalated discs are rarely to be found (Fig. 4A). The myocytes are contracted for the most part. Slight to moderate cellular oedema appears, though non-homogeneously distributed (Fig. 4A, cf. 5A, 5B). Glycogen is abundant and the cell nuclei appear unchanged. Only the mitochondria are clearly swollen (Fig. 4A, cf. 5C, 5D). A few mitochondrial matrix granules are present; the cristae are intact.

After 30 min of pure ischaemia (at 25° C), the interstitial space in the myocyte bundles has already become narrower because of the intracellular oedema of the heart muscle cells. This seldom leads to compression of the capillaries (Fig. 4B). The intercalated discs appear intact. The myocytes are only contracted to about 50% and relaxation has therefore clearly increased. The cell oedema of the myocytes varies from cell to cell and has, on average, greatly increased compared with the initial measurements (Fig. 4B, cf. 5A, 5B). Quantitatively mean values for the glycogen granules are only slightly reduced. The cell nuclei already show evident clumping and increased margination of the chromatin. The mitochondria have lost their matrix granules altogether and show, a moderate or high degree of matrix lightening depending on the extent of the swelling. Furthermore there is real loss of matrix and cristae (Figs. 4B, cf. 5C, 5D).

At the end of HTK perfusion, the ultrastructure of the working myocardium is completely intact (Fig. 4C). Following immersion fixation, the interstitial space has become moderately spread out, the capillaries to varying extents. The unswollen myocytes are either moderately contracted or show narrow I-bands (Fig. 4C, cf. 5A, 5B). Dehiscences of the intercalated discs are not present. The cell nuclei remain unchanged; the dark unswollen mitochondria contain numerous matrix granules (Fig. 4C, cf. 5C, 5D).

Following HTK cardioplegia and 30 min ischaemia at 25° C, the working myocardium shows only small changes in comparison with the initial results (Fig. 4D). The interstitial space and the cellular junctions remain mostly unchanged. The myocytes are homogeneously relaxed, the sarcomeres generally show broader I-bands than at the onset of ischaemia. There is no significant cell oedema, change in the cell nucleus or swelling of the mitochondria compared with initial results (Fig. 4D, cf. 5A–5D). The only obvious effects are a decrease in the number of mitochondrial matrix granules and a slight lightening of the matrix (Fig. 4D).

Morphometrically, cell oedema can be recognised in the change in the volume density of free sarcoplasm (V_{VSp}: Fig. 5A). The differences found

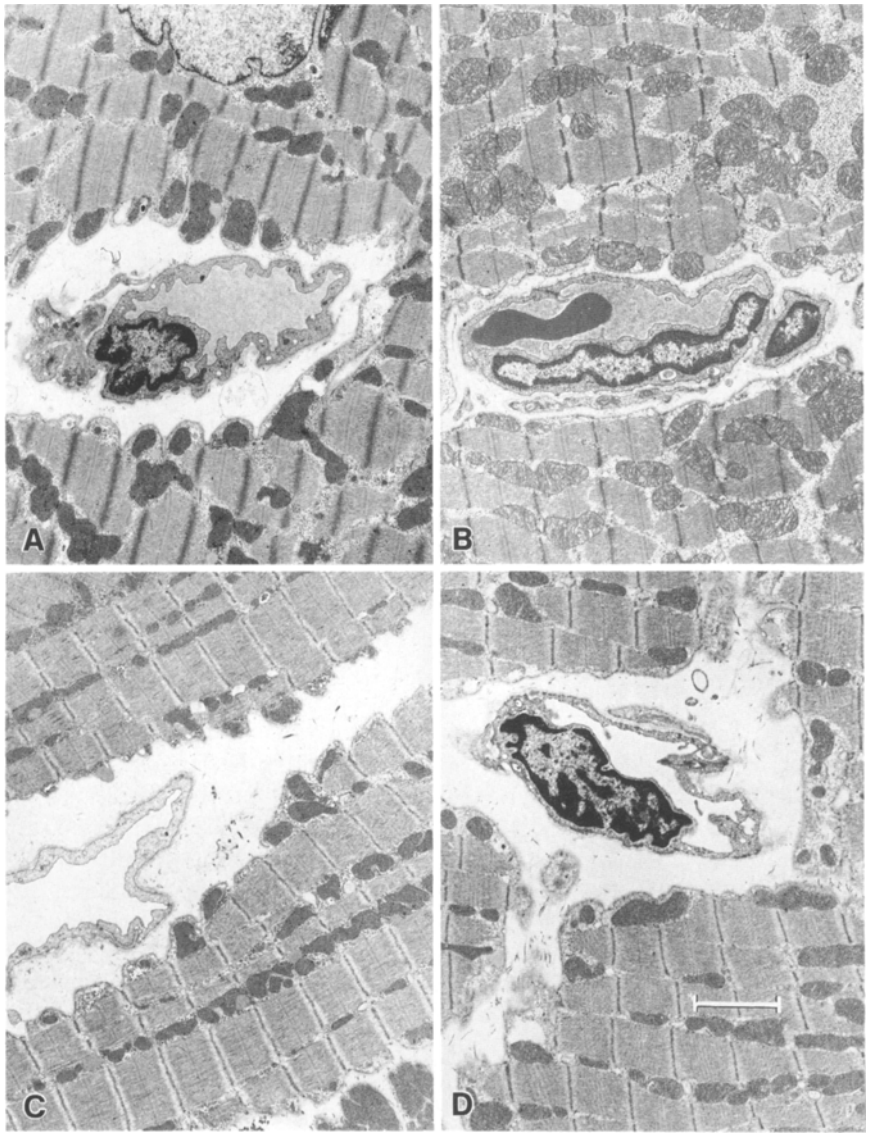

Fig. 4. Ultrastructure of left ventricular working myocardium. **A** pure ischaemia, onset of ischaemia; **B** pure ischaemia, 30 min ischaemia (25° C); **C** HTK cardioplegia, onset of ischaemia; **D** HTK cardioplegia, 30 min ischaemia (25° C) (immersion fixation, × 5600; scale bar: 2 μm)

in the working myocardium are proof of oedema at the onset of pure ischaemia ($8.9 \pm 1.2\%$) with significant ($p \leq 0.05$) progression within 30 min of pure ischaemia ($13.4 \pm 2.4\%$) when compared with HTK cardioplegia ($5.5 \pm 1.3\%$), for which the subsequent value following 30 min ischaemia ($6.2 \pm 1.1\%$) shows no significant increase compared with the initial value.

In the AV nodal cells, conditions are similar to those in the working myocardium. The 30 min of pure ischaemia (V_{VSp}: $42.0 \pm 2.6\%$) leads to serious oedema compared with the same ischaemic stress following HTK cardioplegia (V_{VSp}: $37.8 \pm 2.8\%$).

In the working myocardium, the values for the volume density of myofibrils (V_{VMf}: Fig. 5B) at the onset of ischaemia are significantly ($p \leq 0.05$) lower for pure ischaemia ($68.9 \pm 2.1\%$) than for HTK cardioplegia ($76.3 \pm 1.4\%$). After 30 min of pure ischaemia the decrease in V_{VMf} to a value of $64.8 \pm 1.9\%$ is clearly greater than for HTK cardioplegia where the value at the end of 30 min ischaemia is $74.7 \pm 1.6\%$ ($p \leq 0.01$). After 30 min ischaemia, the AV nodal cells also show a significant ($p \leq 0.05$) difference in V_{VMf} between pure ischaemia ($42.1 \pm 2.6\%$) and HTK cardioplegia ($46.8 \pm 2.8\%$).

In the working myocardium, the volume den-

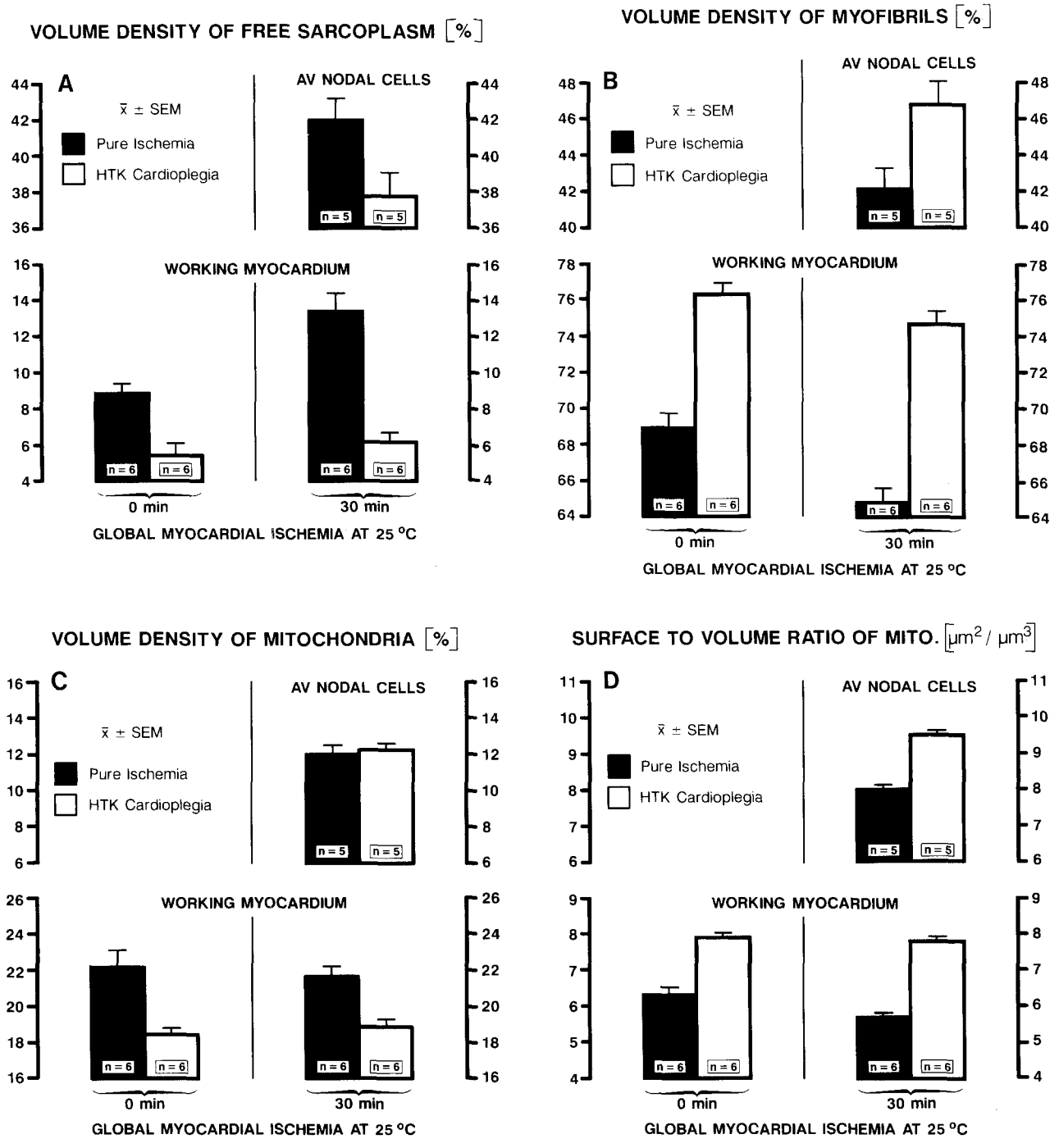

Fig. 5. Morphometry of AV nodal cells and left ventricular working myocardium. **A** Volume density of free sarcoplasm (V_{VSp}). **B** Volume density of myofibrils (V_{VMf}). **C** Volume density of mitochondria (V_{VMi}). **D** Surface to volume ratio of mitochondria ($S_V ratio_{Mi}$)

sity of mitochondria (V_{VMi}: Fig. 5C) is higher both at the onset ($22.2 \pm 2.3\%$) and after 30 min ischaemia ($21.7 \pm 1.3\%$) for pure ischaemia than it is following HTK cardioplegia ($18.5 \pm 0.7\%$) and ensuing ischaemia ($18.9 \pm 1.0\%$). For the AV nodal cells, V_{VMi} is the same for 30 min pure ischaemia ($12.0 \pm 1.0\%$) as for HTK cardioplegia and 30 min ischaemia ($12.3 \pm 0.6\%$).

The surface to volume ratio of mitochondria ($S_V ratio_{Mi}$: Fig. 5D) in the working myocardium at the onset of pure ischaemia ($6.3 \pm 0.5\ \mu m^2/\mu m^3$) is significantly lower ($p \leq 0.01$) than immediately

Table 1. Metabolites in the working myocardium during ischaemia

Ischaemia at 25° C (min)	0	10	30	60
Pure ischaemia ($n=6$)				
Creatine phosphate	46.1±6.6	4.1±1.4	2.7±1.5	–
Adenosine triphosphate	29.9±1.5	25.7±1.6	20.2±2.4	16.7±3.3
Glycogen	384 ±137	417 ±165	388 ±160	354 ±174
Lactate	7.1±7.3	58.9±2.1	78.7±7.2	102 ±12.2
HTK cardioplegia ($n=6$)				
Creatine phosphate	49.5±5.6	42.4±5.0	30.8±7.9	18.2±6.6
Adenosine triphosphate	30.4±7.5	28.3±1.8	27.8±3.0	26.0±2.7
Glycogen	318 ±97	345 ±70	368 ±110	327 ±82
Lactate	4.4±3.8	5.4±3.2	15.7±7.9	33.4±13.5
				$\bar{x}\pm$SD [μmol/g_{DW}]

following HTK cardioplegia ($7.9\pm0.2\ \mu m^2/\mu m^3$). After 30 min of pure ischaemia the value of the $S_V ratio_{Mi}$ decreases further to $5.7\pm0.3\ \mu m^2/\mu m^3$, the value for HTK cardioplegia remaining constant ($7.8\pm0.4\ \mu m^2/\mu m^3$) ($p\leq0.01$). For AV nodal cells, significant differences are seen in the $S_V ratio_{Mi}$ following 30 min ischaemia ($p\leq0.01$), the value being $8.0\pm0.3\ \mu m^2/\mu m^3$ for pure ischaemia and $9.5\pm0.3\ \mu m^2/\mu m^3$ after HTK cardioplegia.

There is hardly any change observed for either method in the volume density of the cell nuclei V_{VCn} in the course of 30 min ischaemia. V_{VCn} lies between 0.5 and 0.9% in the working myocardium, and between 2.2 and 4.0% in the AV nodal cells.

Discussion

Since the investigations reported by Pick (1924), the working myocardium of the left ventricle has been considered to be the "primum moriens" of the heart during myocardial ischaemia. Its metabolite status is regarded as one of the most important factors for the resuscitability of the heart (Bretschneider 1964; Bretschneider et al. 1975, 1983; Jennings and Reimer 1981; Flameng et al. 1984; Schnabel et al. 1987; Gebhard et al. 1989). In Table 1, the tissue concentration of creatine phosphate (CP), adenosine-triphosphate (ATP), glycogen and lactate are reported for the first 60 min of ischaemia at 25° C following aortic cross clamping (pure ischaemia) and HTK cardioplegia.

For each group at the onset of ischaemia, we find physiological initial values per gram dry weight of the left ventricle ($\mu mol/g_{DW}$). Following HTK cardioplegia, compared with pure ischaemia, the CP breakdown, the ATP decay and the increase in lactate are effectively delayed (Gebhard et al. 1987, 1989). The glycogen reserves are hardly affected in either of the groups over the period tested.

The time of ischaemia taken for the CP concentration of the left ventricle to fall from physiological initial values to 15 $\mu mol/g_{DW}$ was designated by Bretschneider (Bretschneider 1964; Bretschneider et al. 1975) t-CP or "survival time." Postischaemic resuscitation within t-CP leads to immediate renewed uptake of function of the heart (Bretschneider 1964; Bretschneider et al. 1975; Gebhard et al. 1989). For pure ischaemia, t-CP is reached after a few min, for HTK cardioplegia after approximately 70 min ischaemia at 25° C (Gebhard et al. 1989).

A reduction in the tissue ATP concentration to about two thirds of the physiological initial values occurs already after approx. 30 min pure ischaemia at 25° C (Bretschneider et al. 1975; Gebhard et al. 1989). The duration of ischaemia necessary to reach this value (corresponding to 20 $\mu mol/g_{DW}$ for the dog) is known as t-ATP (Bretschneider et al. 1975; Gebhard et al. 1989). T-ATP marks the "practical limit of resuscitability" at which the hearts are capable of undertaking the task of circulation without support after a postischaemic resuscitation phase of about 20 min (Bretschneider et al. 1975; Gebhard et al. 1989). For HTK cardioplegia, this limit is reached after about 240 min of ischaemia at 25° C (Gebhard et al. 1987, 1989; Schnabel et al. 1987).

In the case of pure ischaemia, the glycolytic lactate production starts without latency, and the tissue concentration of lactate rises to around 100 $\mu mol/g_{DW}$ within 60 min (Table 1). Following HTK cardioplegia, however, the lactate values start to rise slightly after 30 min, thereafter rising as rapidly as for pure ischaemia (Gebhard et al. 1989). Correspondingly, the development of acido-

sis in the septum is rapidly progressive for pure ischaemia. The interstitial pH ($\bar{x}$; $n=10$) falls from 6.42 after 10 min to 6.02 after 30 min and 5.78 after 60 min pure ischaemia at 25° C (Gebhard et al. 1989). Following HTK cardioplegia ($\bar{x}$; $n=6$), the interstitial pH is equal to 7.57 after 10 min, 6.92 after 30 min and 6.63 after 60 min (Gebhard et al. 1989).

Thus, in the working myocardium of the left ventricle, the acidosis development, lactate production and CP and ATP depletion during the first hour of global ischaemia (at 25° C) differ considerably (Table 1). After 30 min of pure ischaemia the "practical limit of resuscitability" is reached, after HTK cardioplegia and 60 min ischaemia the "survival time" has not been approached.

The metabolic data (Table 1) can be directly compared with the ultrastructural results from the working myocardium, since the samples from the free wall of the left ventricle for the biochemical and for the electron microscopic investigations were always taken parallel to each other. In agreement with the extensive changes in metabolism, for pure ischaemia, the intramural working myocardium shows distinct alterations after 30 min, with swelling of the cells and mitochondria and other damage to fine structure (Figs. 4B, 5A–5D) (Schnabel et al. 1987; Schmiedl et al. 1989b; cf. DiBona and Powell 1980; Jennings and Reimer 1981; Flameng et al. 1984). Following HTK cardioplegia, the biochemical (Table 1) and morphometric changes (Figs. 5A–5D), over the same time period, are only slight. There is a drop in CP tissue concentration and interstitial pH and, ultrastructurally, parallel to this, we see a loss of mitochondrial matrix granules (Fig. 4D) (Schmiedl et al. 1989b).

Only few publications are available on metabolism and ultrastructure of the AV node during ischaemia (Lierse et al. 1974; Thorn et al. 1974). The results for bovine AV nodes, during the first hour of global pure ischaemia, show intensive glycolysis and relatively sudden fall in ATP (Thorn et al. 1974). These changes are accompanied by drastic damage to the fine structure of the AV nodes. Pronounced intracellular and extracellular oedema and a high degree of alteration of the mitochondria, as well as of the contractile system, present further arguments against resistance to ischaemia (Lierse et al. 1974). The strikingly strong intracellular oedema (Fig. 2A) would be the result of a high intensity of glycolysis and the osmotic imbalance thus caused.

Perfusion fixation of the heart was optimised in our preparation by pre-perfusion with the HTK solution (Richter et al. 1984a; Schnabel et al. 1985; Schmiedl et al. 1987), a procedure necessary for examination of the CCS from large hearts by electron microscopy (Schünemann et al. 1988; Clavien 1989; Richter et al. 1989). The results described in this report should be compared with those obtained following HTK perfusion and immediate perfusion fixation as preservation is good (Clavien 1989; Richter et al. 1989; Schmiedl et al. 1989a). The qualitative description of the unaltered ultrastructure in the Results for each type of cell is based on the findings obtained following perfusion fixation as reference (Schnabel et al. 1985; Schmiedl et al. 1987; Clavien et al. 1989b; Richter et al. 1989). For comparison between immersion fixation and perfusion fixation following HTK cardioplegia without ischaemic stress, it can be shown that the cellular parameters are either the same or similar according for the regions under investigation (Schnabel et al. 1985; Schmiedl et al. 1987; Clavien 1989; Clavien et al. 1989a; Richter et al. 1989). Only the interstitial space shows any striking differences. In the working myocardium, the interstitial space in the myocyte bundles is not influenced to such a great extent by the method of fixation as are the tissue clefts separating the myocyte bundles. These are drastically narrowed by perfusion fixation in comparison with immersion (Schmiedl et al. 1989a). The size of the clefts of the layers of loose connective tissue which allows shifting in the region of the subendocardial Purkinje fibres (Doerr and Schiebler 1963; Doerr 1972) is much smaller following perfusion than following immersion fixation. In the superficial part of the compact AV nodes, there are few clefts to be seen following perfusion fixation, the interstitial space appears narrow (Clavien 1989; Clavien et al. 1989b). Following HTK cardioplegia and perfusion fixation, the capillaries take up the greatest part of the interstitial space within the myocyte bundle (Schnabel et al. 1985; Schmiedl et al. 1987, 1989a; Clavien 1989; Richter et al. 1989). The differences in size of the interstitial space are evidence of a particular sensitivity to interstitial oedema in the region of the subendocardial Purkinje fibres and also in the "slack" AV nodes, to which Doerr made particular reference (Doerr 1957, 1959, 1972; Doerr and Schiebler 1963).

Taking the morphometric data following HTK cardioplegia and perfusion fixation shown in Table 2 as particularly well preserved initial values, we can relate the results following 30 min of ischaemia to these data (see Results, Morphometry, Figs. 5A–5D).

For comparison of the degree of cellular oe-

Table 2. Morphometry following HTK perfusion and immediate perfusion fixation

	AV nodal cells $n=3$	Working myocardium $n=9$
Volume density of myofibrils	55.3±1.5	76.1±1.2
Volume density of free sarcoplasm	28.8±1.7	5.3±0.8
Volume density of mitochondria	11.9±0.9	18.1±0.8
Volume density of cell nuclei	4.1±0.6	0.6±0.1
Surface to volume ratio of mitochondria	11.6±0.2	8.4±0.4 $\bar{x}\pm$SD [%]

dema occurring in the different cell types in the ischaemic stress investigated in this study, the volume density of the myofibrils (V_{VMf}: Fig. 5B) appears to be the most suitable parameter. Compared with the data given in Table 2, V_{VMf} is reduced during 30 min of pure ischaemia to varying extents. In the AV nodal cells, it is reduced, for pure ischaemia by 24%, for HTK cardioplegia by 15%, in the working myocardium during pure ischaemia by 15% and after HTK cardioplegia by 2%. During 30 min of ischaemia, none of the tissues investigated showed any evidence of myofibrillolysis. Therefore, V_{VMf} should not change appreciably, as long as the reference space remains constant. However, when the cells swell, V_{VMf} decreases. Assuming the total myofibrils per heart to be constant, V_{VMf} can be taken as a measure of intracellular oedema. In this sense, the development of cell oedema, for pure ischaemia, is proportionally highest for AV nodes, and moderate in the working myocardium. Following HTK cardioplegia, remarkable cellular swelling takes place only in AV nodal cells.

The mitochondrial swelling itself is best judged from the $S_V ratio_{Mi}$, since this parameter for size and shape of mitochondria is independent of the reference space and is not influenced by a simultaneous cellular swelling as is the case for V_{VMi} (Weibel 1979; Mall et al. 1986; Schmiedl et al. 1989b). The $S_V ratio_{Mi}$ decreases with increasing volume of the mitochondria, the surface density remaining constant. This increase of mitochondrial volume is achieved by smoothing of the surface (Schmiedl et al. 1989b). During 30 min of ischaemia, the $S_V ratio_{Mi}$ (Fig. 5D) decreases to differing extents when related to the data in Table 2. The mitochondria in the AV nodal cells swell by 31%, for pure ischaemia, following HTK cardioplegia by 18%, in the working myocardium by 32%, for pure ischaemia and following HTK cardioplegia by 7%. Thus, based on this very sensitive parameter, the swelling of the mitochondria is equally extensive following 30 min of pure ischaemia in the two cell types investigated. In the AV nodal cells following HTK cardioplegia, it is somewhat lessened but still quite obvious, only in the working myocardium is this swelling slight.

These morphometric data and qualitative results clearly show that, based on ultrastructural criteria, the AV nodal cells are not at all resistant to the effects of global ischaemia when compared with the working myocardium of the left ventricle. During 30 min of pure ischaemia at 25° C, intracellular oedema is markedly more pronounced in the AV nodal cells. The swelling of the mitochondria is equally extensive in both tissues. Following HTK cardioplegia and under the same ischaemic stress, the changes in fine structure are, in all, markedly less pronounced than for pure ischaemia. They show a tendency, however, to be weaker in the working myocardium compared with the AV node.

We cannot conclude that tolerance of ischaemia is lower in all structures of the CCS. It is even possible that the AV nodes and the terminal parts of the ventricular Purkinje system (Richter et al. 1986; Schnabel et al. 1988), may be more sensitive to certain noxae (Opie 1985) in contrast with the type I Purkinje fibres in the His-bundle (Armiger and Knell 1986). In spite of the caution necessary in a comparative judgement of the changes in structure and function (Doerr 1959; Doerr and Schiebler 1963; Friedman et al. 1975) extracellular oedema may through a slackening of the cell connections, provide an explanation for certain reversible disturbances in the cardiac conduction system, such as those encountered following global ischaemia without surgical lesions (Coffman et al. 1960; Bagdonas et al. 1961; Adappa et al. 1978; Smith et al. 1983; Flameng et al. 1984; Scheld 1984; Opie 1985; Niederberger von Wolfenschiessen 1987). The intracellular changes following pure ischaemia, which are considerable, may be seen as the cause of serious, reversible or irreversible rhythm disturbances. In the case of HTK cardioplegia, the successful protection of the structure of the working myocardium and the cells of the CCS can be correlated with a complete reversibility of the changes and a rapid postischaemic recovery of function (Gebhard et al. 1984; Scheld 1984; Schnabel et al. 1988; Walter et al. 1988).

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