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# The ultrastructural effects of global ischaemia on Purkinje fibres compared with working myocardium: a qualitative and morphometric investigation on the canine heart

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**Summary.** During open heart surgery, reperfusion-induced arrhythmias arising after short periods of ischaemia may originate from subendocardial Purkinje fibres. We investigated the ultrastructure of these fibres during 30 min of global ischaemia at 25° C. The effects both with myocardial protection (HTK cardioplegia) and without it (pure ischaemia) were compared qualitatively and morphometrically. After 30 min pure ischaemia overcontraction of sarcomeres, hypercontraction and contraction bands, together with considerable changes in organelles, predominate over cellular oedema. In Purkinje fibres, both cellular and mitochondrial swelling were significantly increased within this 30-min time period from the onset of pure ischaemia. In contrast, following HTK cardioplegia and 30 min ischaemia, cellular and mitochondrial swelling remain moderate and overcontractions are almost entirely lacking. This means that despite remarkable differences between pure ischaemia and HTK cardioplegia in the degree of protection attained it is clear that, compared with the working myocardium, subendocardial Purkinje fibres do not display a higher resistance to early global ischaemia. Further investigations of this sensitivity of Purkinje fibres to global ischaemia and certain drugs may bring about new insights into myocardial protection and pharmacotherapy of arrhythmias.

**Key words:** Purkinje fibres – Ischaemia tolerance – Qualitative and quantitative ultrastructure – Cardioplegia – Arrhythmias

## Introduction

The ischaemic tolerance of different parts of the cardiac conduction system (CCS) is debated, as are its fine structural criteria (see Armiger and Knell 1988; Tribulova et al. 1988; Schnabel et al. 1990a). From experiments on cyanide poisoning and post-ischaemic revivability, Pick (1924) concluded that the Purkinje fibres appeared to represent the *ultimum moriens* of the heart functionally. The popular assumption of a higher tolerance to ischaemia was supported by the results of regional ischaemia, demonstrated by the clinical effects following myocardial infarction, where it appeared that the working myocardium was more often and more seriously affected than the CCS. The subendocardial Purkinje fibres are supplied with oxygen via the terminal vascular bed and by diffusion from the endocardium. Electrophysiological and ultrastructural findings indicate that in spite of (or because of) their exposed position, they might be the source of certain forms of arrhythmias following myocardial infarction (Friedman et al. 1975; Lurie et al. 1987).

In contrast, following global ischaemia new postoperative conduction disturbances including ventricular arrhythmias can be related to a greater susceptibility of parts of the CCS to the impact of ischaemia and to intraoperative measures (Michelson et al. 1979; Pattison et al. 1988; Schnabel et al. 1990a). Depending on the ambient conditions, however, gas exchange in the surface layers will not be completely suspended (Bretschneider 1961; Henry and Lowry 1985; Lurie et al. 1987; Gebhard and Bretschneider 1989).

Recently, qualitative electron microscopical investigations have shown rather different alterations in subendocardial Purkinje fibres and working myocardium due to global ischaemia, effects which relate to the method of cardiac arrest (Richter et al. 1986; Schnabel et al. 1988a, b; Ramsauer et al. 1989). Many descriptions of Purkinje fibres of different species or of special structural details have been given (see Schiebler 1953; Doerr 1957; Kawamura 1961; Truex and Smythe 1965; Martinez-Palomo et al. 1970; Thornell et al. 1976; Armiger et al. 1979; Sommer and Dolber 1982; Canale et al. 1983; Richter et al. 1986; Viragh et al. 1987; Schnabel et al. 1988a, 1990b). For different species, quantitative or

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morphometric data on Purkinje fibres without ischaemic stress are rather sparsely documented (Muir 1957; Pape et al. 1969; Mobley and Page 1972; Sommer and Dolber 1982; Eisenberg and Cohen 1983). Systematic comparative studies of ischaemic alterations in different regions of the CCS in the same species (cf. Armiger and Knell 1988; Tribulova et al. 1988; Schnabel et al. 1990a) have also rarely been available.

We therefore analysed the ultrastructure of the subendocardial Purkinje fibres (type II according to Viragh et al. 1987) in comparison with the left ventricular working myocardium in the canine heart following 30 min of global ischaemia at 25° C. Both qualitative and morphometrical studies were made. The results with myocardial protection (HTK cardioplegia) and without it (pure ischaemia) were compared with reference values obtained without ischaemic stress.

## Materials and methods

As described previously (Schnabel et al. 1987a; Schmiedl et al. 1990a), artificial cardiac arrest was induced in mongrel dogs of both sexes (26–33 kg) in combined neurolept-analgesia (Kehrer et al. 1989) by inflow occlusion, aortic cross-clamping and for pure ischaemia ($n=5$) by topical cooling with ice-cold Tutofusin solution (composition in mmol/l: 140 NaCl, 5 KCl, 2.5 $CaCl_2$, 1.5 $MgCl_2$; Pfrimmer, Erlangen, FRG), and for HTK cardioplegia ($n=5$) by 11 min coronary perfusion with cardioplegic solution HTK (Bretschneider) at 8° C (composition in mmol/l: 15 NaCl, 9 KCl, 4 $MgCl_2$, 180 *h*istidine, 18 histidine-HCl, 2 *t*ryptophan, 1 K-α-*k*etoglutarate, 30 mannitol; Koehler, Alsbach, FRG).

Samples were taken in situ at the onset of ischaemia and following incubation of the left ventricle in the solution employed for cardiac arrest after 30 min of global ischaemic at 25° C. Samples from the subendocardial third (approx. 5–10 mm) were cut with scissors and fixed by immersion. Immediately after immersion in the fixative, smaller tissue blocks (approx. 1 $mm^3$) for light and electron microscopical investigations were dissected with razor blades so that the relationship between the subendocardial Purkinje fibres and the immediate neighbouring working myocardium (Fig. 1) remained intact as far as possible during fixation (Richter et al. 1986; Schnabel et al. 1988a).

Additionally, a group of hearts was fixed by perfusion fixation immediately after the end of HTK cardioplegia ($n=5$) produced by 11 min coronary perfusion with HTK solution and subsequent 15 min perfusion fixation (Schnabel et al. 1987b, 1990a; Schmiedl et al. 1990b). These hearts were dissected by scalpel.

Fixation and further processing of the tissue blocks, sectioning and sampling have been described recently (Schmiedl et al. 1990a; Schnabel et al. 1990a). Three random sections of three random tissue blocks were evaluated per heart and time point. All qualitative and morphometrical measurements were carried out with an EM 10 (Zeiss, Oberkochen, FRG) equipped with a TV camera. Following systematic random sampling (Weibel 1979), 50 test fields per section were measured for the morphometry of the Purkinje fibres (Schnabel et al. 1990a).

The contraction state of the sarcomeres was ascertained as described elsewhere (Schnabel et al. 1990b). For the morphometry, only those parts of the sections in which all substructures can be recognised unequivocally and defined can be used. Since, in the Purkinje fibres, the irregular hypercontractions and contraction bands lead to serious damage of the ultrastructure, the complete morphometrical analysis of this type of area is not possible. The ratio of these areas to the respective cells as a whole was quantified.

Based on the point-counting system of Weibel (1979), at a final magnification of ×45500, the volume densities ($V_V$) of the myofibrils ($V_{VMf}$), the free sarcoplasm ($V_{VSp}$), the mitochondria ($V_{VMi}$) and the cell nuclei ($V_{VCn}$) were measured using the cells as reference space (Schmiedl et al. 1990a; Schnabel et al. 1990a). Furthermore the surface to volume ratio of mitochondria ($S_V$ $ratio_{Mi}$) was determined as a parameter for form and size of mitochondria independent of the reference space, using the method of Weibel (1979) (Mall et al. 1988; Schmiedl et al. 1990a).

All results are given as mean values ±SEM unless indicated otherwise. Significant differences have been noted for all $P$ values of 0.05 or less using the Wilcoxon-Mann-Whitney (U) test, for unpaired samples or the Student's $t$-test for paired samples.

## Results

In the region of transition from the terminal part of the CCS to the working myocardium, in the subendocardial position, three main cell types can usually be distinguished (Fig. 1): Purkinje fibres, transitional cells and subendocardial working myocardium. Below the endocardium, either smaller bundles of Purkinje fibres of a mean diameter of 20–30 µm are found (Fig. 1, right), or there are one to three layers of these Purkinje fibres as a subendocardial network generally surrounded by connective tissue (Fig. 1, left). These are followed by a layer of very narrow transitional cells (mean diameter 6–10 µm) which can often not be clearly identified by light microscopy or which are not cut in the semithin sections. In the overall view, a relatively wide interstitial space in the region of the subendocardial Purkinje fibres is evident and this is partially due to immersion fixation (Fig. 1).

The end-to-end connections of the Purkinje fibres have more desmosomes and gap junctions and thus fewer undifferentiated areas than the cellular junctions of the AV nodal cells. They are, however, much fewer interlocked than intercalated discs in the working myocardium (Fig. 2, cf. Schnabel et al. 1990a). Furthermore, the Purkinje fibres are connected by lateral contacts which are also rich in gap junctions. These fibres have mostly large, more centrally localized areas rich in glycogen and ribosomes which can be distinguished quite easily from other areas in the overall view because they appear very light in colour (Fig. 1). These large glycogen-rich areas are not always found, mainly depending on the depth at which the cells are cut. Apart from filaments and filament-ribosome complexes, they also show mitochondria in marginal positions. Morphometrically, these large "glycogen pools" contribute about 25% to the free sarcoplasm of the subendocardial Purkinje fibres, whereas nearly 75% are found in intermyofibrillar position (Figs. 2, 3A–D, cf. Fig. 4B). The myofibrils are not as regularly arranged as in the working myocardium and build a three-dimensional network within the cells in the directions of the Purkinje fibre network, so that they are seldom found longitudinally over the whole length of a cell (Figs. 2, 3A–D, cf. Fig. 4B). The cell nuclei occupy only a very small, usually central part of the Purkinje fibres; the chromatin is very finely distributed without clumping or margination (Fig. 2). The mitochondria appear strikingly smaller and narrower than in the working myocardium (Figs. 2, 3A, C, D,

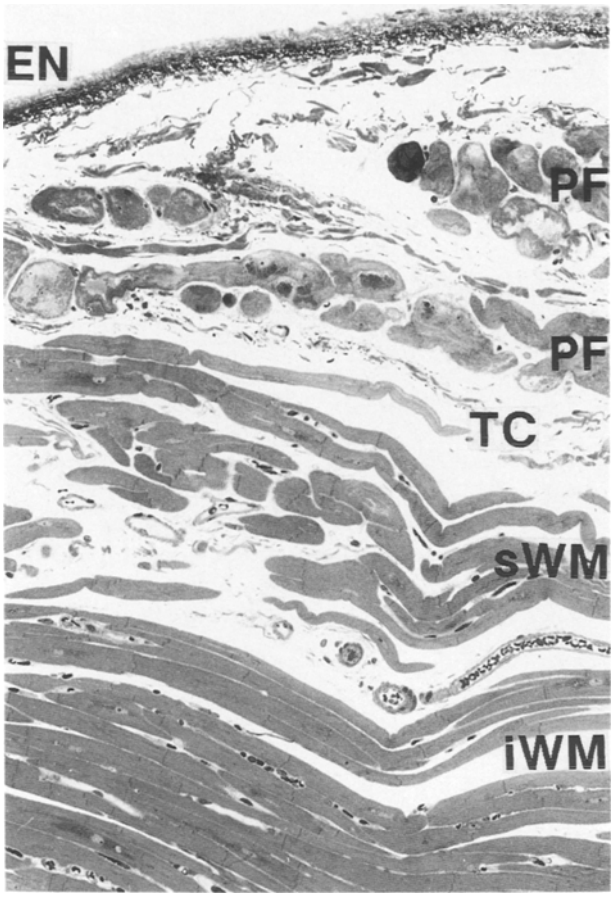



**Fig. 1.** Subendocardium of the left ventricle; pure ischaemia, onset of ischaemia. *EN*, Endocardium with endothelium and endocardial connective tissue; *PF*, Purkinje fibres; *TC*, transitional cells; *sWM*, subendocardial working myocardium; *iWM*, intramural working myocardium. Immersion fixation, methylene blue, azure II, ×200

cf. Fig. 4B, C) and they are sometimes very long and branched (Fig. 3C, D). Like the AV nocal cells, Purkinje fibres do not possess a T-system.

In transitional cells, however, transversal tubules may be present in the canine heart. In a continuous layer of transitional cells, only separated by an intercalated disc, one can sometimes find a cell showing the characteristics of the CCS without T-tubules and a cell with the typical characteristics of the working myocardium and unmistakable T-tubules. Normally, the ratio of Purkinje fibres to transitional cells in a subendocardial network is 5:1, less often 5:2.

At the onset of pure ischaemia, immediately following aortic cross-clamping, the Purkinje fibres are, for the most part, contracted (Fig. 3A). The relatively wide interstitial space and the cell contacts of the Purkinje fibres show no changes. Through the partially homogeneous shortening of the sarcomeres, the fibres become thicker in cross-section, the individual cells shortened. Intracellular oedema is not seen. The fraction of the free sarcoplasm is less than that of the contracted myofibrils, which appear dominant (Fig. 3A). The cell nuclei show the typical chromatin distribution. Most of the mitochondria appear dark, displaying only slight matrix lightening or loss of their typical granules. Other fine structural details of the mitochondria remain unaltered (Fig. 3A, cf. Fig. 4A–C).

After 30 min pure ischaemia at 25° C, the ultrastructure of the Purkinje fibres undergoes marked alteration. The interstitial space is hardly altered and the cell contacts only show changes in regions of extreme overcontraction or contraction bands. In Purkinje fibres and particularly in transitional cells, inhomogeneous contractions, overcontractions and contraction bands of the sarcomeres dominate (Fig. 3B, cf. Fig. 4A, B). Even in

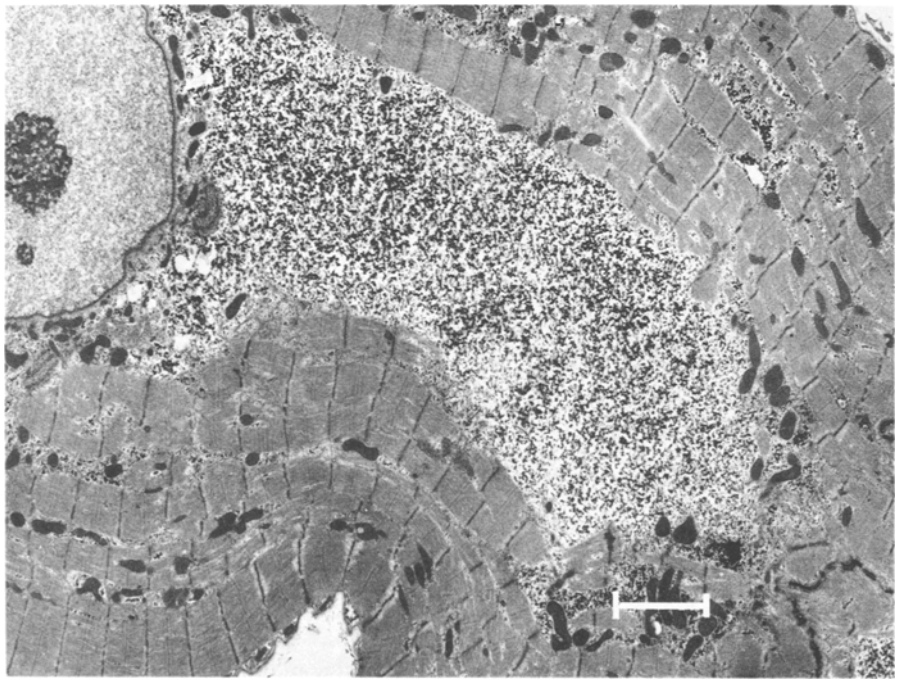


**Fig. 2.** Ultrastructure of a subendocardial Purkinje fibre of the left ventricle, immediately after HTK perfusion and subsequent perfusion fixation. ×5600. *Scale bar* = 2 μm

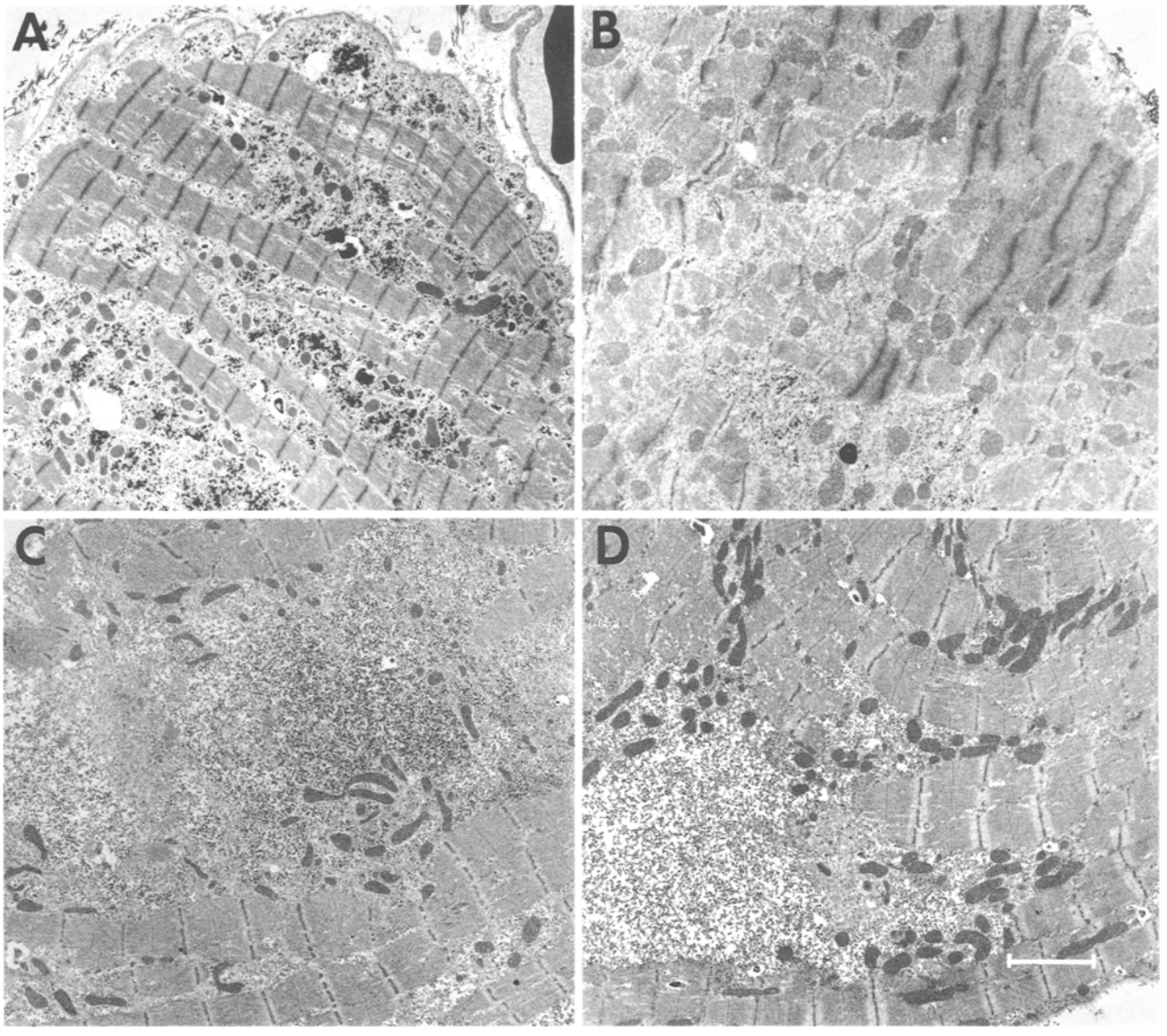
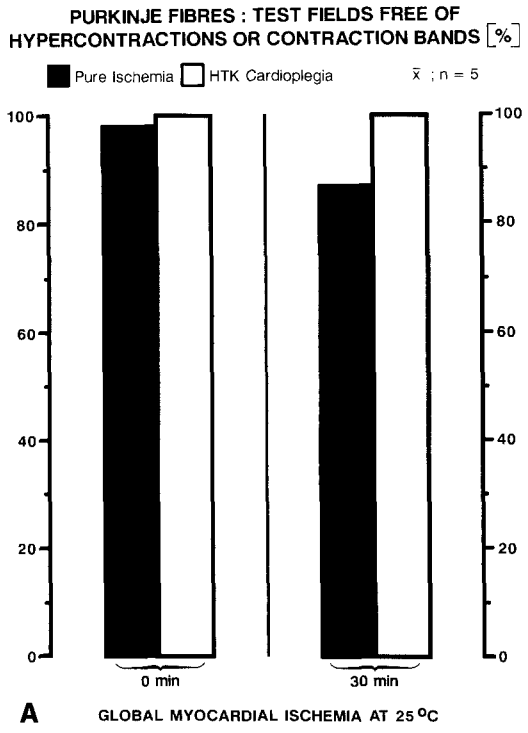


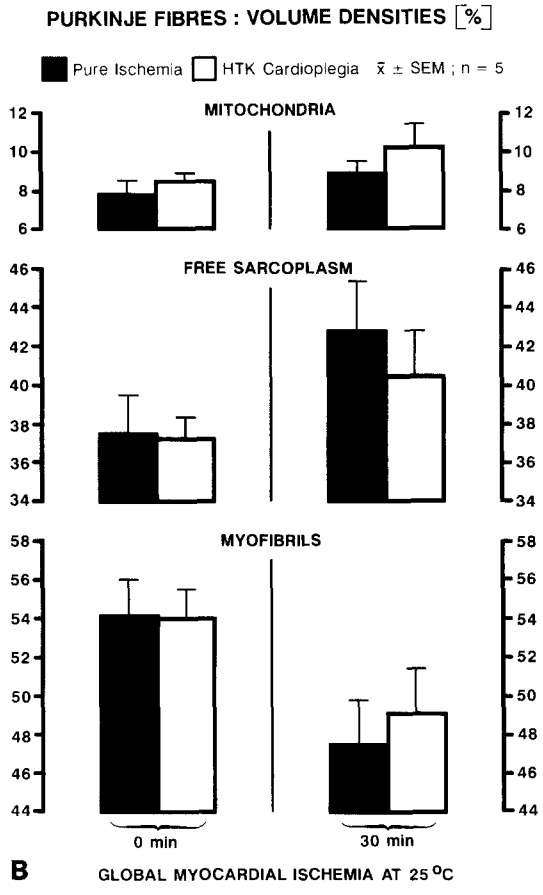

**Fig. 3A–D.** Ultrastructure of subendocardial Purkinje fibres of the left ventricle. **A** Pure ischaemia, onset of ischaemia; **B** pure ischaemia, 30 min ischaemia (25° C); **C** HTK cardioplegia, onset of ischaemia; **D** HTK cardioplegia, 30 min ischaemia (25° C). Immersion fixation, $\times 5600$; *scale bar* = 2 μm

strongly contracted Purkinje fibres, intracellular oedema is seen under the sarcolemma and also between the myofibrils. This is not true for the irregularly overcontracted parts (Fig. 3B). The glycogen appears to be reduced, in some areas dissolved out, so that sometimes small empty areas appear in the sarcoplasm (Fig. 3B, cf. Fig. 4A, B). Isolated vacuoles coated with membrane probably arise from dilated sarcoplasmic reticulum. The mitochondria are rather swollen (Fig. 3B, cf. Fig. 4A–C). They seldom contain single matrix granules but reveal a clear loss of matrix structure (cf. Fig. 3B with 3A). Furthermore, the cristae mitochondriales show fragmentation and small areas of cristolysis.

After initiation of HTK cardioplegia, at the onset of ischaemia, the Purkinje fibres look different from their appearance immediately following aortic cross-clamping (Fig. 3C). The fibres appear relaxed showing narrow I-bands or are contracted. Interstitial space and cell contacts appear unchanged. Areas of free sarcoplasm of varying sizes in which glycogen granules are finely distributed are spread out among the cords of the myofibrils (Fig. 3C, cf. Fig. 4A, B). Like the Purkinje fibres themselves, the cell organelles appear unchanged and are not swollen (Fig. 3C, cf. Fig. 4A–C).

After HTK cardioplegia and 30 min of ischaemia at 25° C, only slight changes can be observed in the Purkinje fibres, compared with the initial values (Fig. 3D). The contractile system appears partly contracted and partly relaxed; broader I-bands can be seen in longitudinal sections of the latter (Fig. 3D, cf. Fig. 4A, B). The free sarcoplasm has, in general, slightly increased; the glycogen granules and ribosomes are no longer completely homogeneously distributed (Fig. 3D, cf. Fig. 4A, B). Loss of glycogen is, for the most part, not observed. The nuclear chromatin shows neither clumping nor increased margination. In the majority of the Purkinje



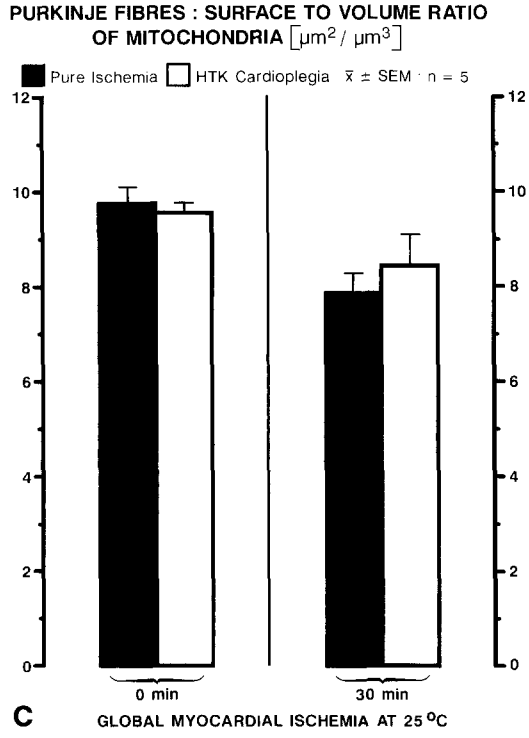




**Fig. 4A–C.** Morphometry of the subendocardial Purkinje fibres of the left ventricle. **A** Percentage of test fields which could be evaluated morphometrically (test fields showing hypercontractions or contraction bands had to be excluded because of severe ultrastructural damage). Thus, the values for pure ischaemia, which represent only the remaining areas where morphometry was possible, are positive selections of the total population of the Purkinje fibres. **B** Volume densities of mitochondria ($V_{vMi}$), free sarcoplasm ($V_{vSp}$), and myofibrils ($V_{vMf}$). **C** Surface to volume ratio of mitochondria ($S_V ratio_{Mi}$)

fibres, a slight to moderate swelling of the mitochondria is to be found (Fig. 3D, cf. Fig. 4A–C). Sometimes marked swelling with a lighter matrix is seen but there is no fragmentation of the cristae.

The qualitative and morphometric descriptions of the intramural working myocardium and the AV nodal cells have been reported recently (Schnabel et al. 1990a). In general, the changes in the working myocardium dur-

ing global ischaemia are similar subendocardially and intramurally, though their appearance is somewhat delayed in the subendocardium.

The parts of the Purkinje fibres which could not be morphometrically evaluated due to the presence of hypercontractions or contraction bands account for 2% of the Purkinje fibres at the onset of pure ischaemia and for 13% after 30 min (Fig. 4A). These forms of contraction lead to such severe ultrastructural damage that substructures can no longer be identified. In contrast, following HTK cardioplegia and subsequent 30 min ischaemia, all of the Purkinje fibres could be evaluated (Fig. 4A). The morphometric results are given as mean values plus/minus standard deviation.

The relative decrease of $V_{VMf}$ (Fig. 4B) under these conditions can be regarded as an indicator of cellular oedema (Schnabel et al. 1990a). In the Purkinje fibres, $V_{VMf}$ drops significantly from $54.1 \pm 4.3\%$ to $47.5 \pm 4.9\%$ during 30 min of pure ischaemia ($P < 0.02$). For HTK cardioplegia the decrease in $V_{VMf}$ is less pronounced over the same period of time ($54.0 \pm 3.5\%$ as opposed to $49.1 \pm 5.2\%$).

In the Purkinje fibres, the large standard deviations in $V_{VSp}$ (Fig. 4B) can be explained in part by the varying numbers of large glycogen-rich areas. The increase in $V_{VSp}$ during 30 min of pure ischaemia from $37.5 \pm 4.4\%$ to $42.8 \pm 5.7\%$ is remarkable ($P < 0.02$). Following HTK cardioplegia, the increase in $V_{VSp}$ from $37.2 \pm 2.6\%$ to $40.5 \pm 5.1\%$ is moderate.

The $V_{VMi}$ (Fig. 4B) is influenced both by cellular and mitochondrial oedema. The Purkinje fibres show a slight to moderate increase in $V_{VMi}$ during the first 30 min of ischaemia – for pure ischaemia from $7.8 \pm 0.5\%$ to $8.8 \pm 1.6\%$, after HTK cardioplegia from $8.5 \pm 1.0\%$ to $10.2 \pm 2.8\%$.

The $S_v ratio_{Mi}$ (Fig. 4C) is a parameter for mitochondrial swelling independent of the reference space (Schmiedl et al. 1990a). During 30 min of pure ischaemia, the $S_v ratio_{Mi}$ for the Purkinje fibres shows a striking drop from $9.8 \pm 0.8$ to $7.9 \pm 0.9$ $\mu m^2/\mu m^3$ ($P < 0.05$). For the same time period, the changes following HTK cardioplegia from $9.5 \pm 0.6$ to $8.5 \pm 1.5$ $\mu m^2/\mu m^3$ are obviously less pronounced.

There is hardly any change in $V_{VCn}$ observed for either method in the course of 30 min ischaemia. $V_{VCn}$ lies between 0.3 and 0.9% in the Purkinje fibres.

## Discussion

The striking ultrastructural alterations of the Purkinje fibres during 30 min of pure ischaemia are not only pronounced when compared with the subendocardial working myocardium, but also when compared with the intramural working myocardium (Schnabel et al. 1990a). The differences in the contraction state of the sarcomeres are most obvious; they are discussed in detail elsewhere (Schnabel et al. 1990b) and are summarized here briefly. The definitions of the different contraction states, the estimation of reversibility and the results for the intramural working myocardium during ischaemia are in agreement with the literature (Vanderwee et al. 1981; Ganote 1983; Karch and Billingham 1985). Published data concerning the subendocardial Purkinje fibres, transitional cells and neighbouring working myocardium are few. In our experiments, physiological contraction states (relaxation = I-bands present; contraction = I-bands absent) were discriminated electron microscopically from pathological states (overcontraction = A-band compression; hypercontraction = actin-myosin clumping; contraction band = actin-myosin clumping plus rupture of the contractile system) (Schnabel et al. 1990b). The occurrence of contraction bands after 30 min of pure ischaemia is restricted to the superficial layers and is completely prevented by HTK cardioplegia. In these layers residual mitochondrial energy production, required for contraction banding (Vander Heide et al. 1986), is possible because of oxygen diffusion (Bretschneider 1961; Henry and Lowry 1985; Lurie et al. 1987; Gebhard and Bretschneider 1989). Nevertheless, the formation of contraction bands (and of hypercontractions) is most possibly due to the effects of at least one noxa associated with ischaemia – or combined effects of several noxae, for example catecholamines, calcium, oxygen metabolites or glycolytic products (Singal et al. 1983; Neely and Grotyohann 1984; Karch and Billingham 1985).

A particular sensitivity of the Purkinje fibres to acidosis can bring about triggered activity even in mild hypoxia (Adamantidis et al. 1986) and the changes observed may be related to this. Interstitially, after 30 min of pure ischaemia, a pH value of about 6.0 is reached (Schnabel et al. 1990a). This critical pH value is noteworthy since investigations on the ultrastructure of the capillary endothelial cells of the heart have shown that (independent of the method of cardiac arrest) the morphometrically measured mean endothelial cell thickness only increases substantially if the interstitial pH falls below 6.0 (Pomykaj et al. 1986; Schnabel et al. 1987a; Bretschneider et al. 1988). Following post-ischaemic resuscitation persistant endothelial swelling brings about disturbances of the microcirculation. Under certain circumstances this occurs in combination with contraction bands (Richter et al. 1984; Anderson et al. 1987; Schnabel et al. 1987a).

Under normal conditions the contribution of anaerobic glycolysis to a lower energy demand of Purkinje fibres is relatively higher than in the working myocardium where oxidative phosphorylation dominates (see Schiebler 1953; Pape et al. 1969; Kübler et al. 1985; Schnabel et al. 1990a). Despite this lower metabolic rate of Purkinje fibres and despite at least partial supply of their oxygen demand by diffusion, there are no significant differences in the decline of energy rich phosphates between subendocardial Purkinje fibres and adjacent ventricular muscle cells following 24 h myocardial infarction in the canine heart (Lurie et al. 1987). Moreover, in the human heart, an obvious depletion of the glycogen reserves of the Purkinje fibres is the most important finding following acute ischaemia (Elias et al. 1982). These results are in agreement with our present ultrastructural findings, which clearly show that at the

**Table 1.** Morphometry following HTK perfusion and immediate perfusion fixation

| | Purkinje fibres $n=5$ | Working myocardium $n=9$ | |
|---|---|---|---|
| Volume density of myofibrils | 53.4±5.8 | 76.1±1.2 | (%) |
| Volume density of free sarcoplasm | 40.2±6.0 | 5.3±0.8 | (%) |
| Volume density of mitochondria | 6.0±0.7 | 18.1±0.8 | (%) |
| Volume density of cell nuclei | 0.4±0.2 | 0.6±0.1 | (%) |
| Surface to volume ratio of mitochondria | 11.1±0.9 | 8.4±0.4 $\bar{x}\pm$SD | ($\mu m^2/\mu m^3$) |

onset of ischaemia the working myocardium profits much more from cardioplegia and hypothermia (which both lower the metabolic rate) than the Purkinje fibres. During global ischaemia the protective efficacy is higher in the working myocardium during pure ischaemia and HTK cardioplegia (Schnabel et al. 1990a, b).

Taking the morphometric data following HTK cardioplegia and immediate perfusion fixation shown in Table 1 as particularly well preserved initial values (Schnabel et al. 1987b; Schmiedl et al. 1990b) we can relate the results following 30 min of ischaemia to these data and the data of the working myocardium (Fig. 4B, C, cf. Schnabel et al. 1990a).

For the comparison of the degree of cellular oedema occurring in the Purkinje fibres for the ischaemic stress investigated in this study, the $V_{VMf}$ (Fig. 4B) is the most suitable parameter (Schnabel et al. 1990a), since during 30 min of ischaemia, none of the cells investigated morphometrically showed any evidence of myofibrillolysis. Purkinje fibres showing hypercontractions or contraction bands had to be excluded from the morphometry (see Results). Compared with the data for optimal preservation (Table 1), $V_{VMf}$ is reduced during 30 min of ischaemia to varying extents, i.e. in the remaining Purkinje fibres for pure ischaemia by 11%, for HTK cardioplegia by 8%, in the working myocardium during pure ischaemia by 15% and after HTK cardioplegia by 2% (Schnabel et al. 1990a). This constellation can be explained by differences in the metabolic rate and in the contraction state. Firstly, glycolysis in the working myocardium starts more rapidly during pure ischaemia than during HTK cardioplegia (Schnabel et al. 1990a), possibly being somewhat more extensive than in the Purkinje fibres (cf. Schiebler 1953; Elias et al. 1982; Henry and Lowry 1985; Kübler et al. 1985; Lurie et al. 1987). Secondly, the stronger contraction in Purkinje fibres compared with the working myocardium (Schnabel et al. 1990b) might provide mechanical limitations to cellular swelling (Pine et al. 1979; Schnabel et al. 1988b).

The mitochondrial swelling itself is best represented by the $S_v ratio_{Mi}$ (Weibel 1979; Mall et al. 1988; Schmiedl et al. 1990a). During 30 min of ischaemia, the $S_v ratio_{Mi}$ (Fig. 4C) decreases to differing extents depending on experimental conditions. Compared with the data in Table 1, the decrease in the $S_v ratio_{Mi}$ for the remaining Purkinje fibres during pure ischaemia is 29%, following HTK cardioplegia 24%. In the case of the working myocardium the decrease of $S_v ratio_{Mi}$ is 32% for pure ischaemia and 7% following HTK cardioplegia (Schnabel et al. 1990a). These differences might be due to mitochondrial preservation of transmembrane potential, ion and especially proton gradients during ischaemia (Andersson et al. 1987; Stier et al. 1989). This function is probably more important for the Purkinje fibres, as their smaller surface area per unit volume and the complete lack of a T-system render the exchange over the sarcolemma more difficult than for the working myocardium. Besides this, there is a strikingly smaller $V_{VMi}$ in the Purkinje fibres (see Results, cf. Table 1). Moreover, in pure ischaemia the most severely swollen mitochondria of the Purkinje fibres had to be excluded from the morphometry.

The qualitative and quantitative results given in this study and also those published recently (Schnabel et al. 1990a) clearly show that, based on ultrastructural criteria, the parts of the CCS under investigation 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, the intracellular oedema is most markedly pronounced in the AV nodal cells. In the Purkinje fibres, occurring together with substantial overcontractions or contraction bands of the sarcomeres, it appears to be less than in the working myocardium. The swelling of the mitochondria is equally extensive in all three tissues. Following HTK cardioplegia and under the same ischaemic stress, pathological overcontractions are almost entirely lacking. The changes in the fine structure are, in all, markedly less pronounced than for pure ischaemia. Nevertheless, the structural protection appears to be somewhat more effective in the working myocardium compared with areas of the CCS.

Some important conclusions can be drawn from these unexpected results:

1. The lower metabolic rate of cells of the CCS under normal conditions (Schiebler 1953; Elias et al. 1982; Henry and Lowry 1985; Kübler et al. 1985; Lurie et al. 1987) does not mean that these cells are necessarily better preserved in pathophysiological situations such as ischaemia, when compared to the working myocardium. Assuming a similar basal metabolic rate for the different myocardial tissues (Singer and Bretschneider 1990), the efficiency of methods for myocardial protection may be less for cells of the CCS, since the margin available before reaching a critically lowered metabolic rate is smaller than for the working myocardium.
2. During ischaemia, cardioplegia should not only preserve metabolism, (high levels of energy-rich phosphates) but also prevent structural damage by an appropriately balanced electrolyte composition in combination with high concentrations of an amino acid buffer (Bretschneider 1980; Schnabel et al. 1987a; Bretschneider et al. 1988; Gebhard and Bretschneider 1989; Gebhard and Schnabel 1989; Kehrer et al. 1989).
3. The striking ultrastructural alterations of the contrac-

tile system up to contraction bands during the early phase of global ischaemia (cf. Schnabel et al. 1988a, 1990a, b; Ramsauer et al. 1989) could be seen as a reason for the early arrhythmias (Hearse 1988) which occur during short periods of otherwise reversible ischaemia.
4. The sensitivity of the Purkinje fibres to the impacts of global ischaemia can also be seen parallel to a certain sensitivity to some drugs such as glycosides (Doerr 1957; Reiser and Anderson 1981) or anti-arrhythmics which prevent arrhythmias in normal and produce arrhythmias in ischaemic Purkinje fibres (Gough and El-Sherif 1989). Thus, further experiments on ischaemia tolerance of parts of the CCS should not only bring insight into aspects of myocardial protection in cardiac surgery but also into the pharmacotherapy of arrhythmias in the wider sense.

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## References

Adamantidis MM, Caron JF, Dupuis BA (1986) Triggered activity induced by combined mild hypoxia and acidosis in guinea-pig Purkinje-fibers. J Mol Cell Cardiol 18:1287–1299

Anderson PG, Bishop SP, Digerness SB (1987) Transmural progression of morphologic changes during ischemic contracture and reperfusion in the normal and hypertrophied rat heart. Am J Pathol 129:152–167

Andersson BG, Aw TY, Jones DP (1987) Mitochondrial transmembrane potential and pH gradient during anoxia. Am J Physiol 252:C349–C355

Armiger LC, Knell CM (1988) Fine structural alteration in the atrioventricular junctional conduction tissues of the dog heart during severe ischemia. J Submicrosc Cytol Pathol 20:645–656

Bretschneider HJ (1961) Sauerstoffbedarf- und versorgung des Herzmuskels. Verh Dtsch Ges Kreislaufforsch 27:32–59

Bretschneider HJ (1980) Myocardial protection. Thorac Cardiovasc Surgeon 28:295–302

Bretschneider HJ, Helmchen U, Kehrer G (1988) Nierenprotektion. Klein Wochenschr 66:817–827

Canale E, Campbell GR, Uehara Y, Fujiwara T, Smolich JJ (1983) Sheep cardiac Purkinje fibers: configurational changes during the cardiac cycle. Cell Tissue Res 232:97–110

Doerr W (1957) Die Morphologie des Reizleitungssystems, ihre Orthologie und Pathologie. In: Spang K (ed) Rhythmusstörungen des Herzens. Thieme, Stuttgart, pp 1–46

Eisenberg BR, Cohen IS (1983) The ultrastructure of the cardiac Purkinje strand in the dog: a morphometric analysis. Proc R Soc Lond B 217:191–213

Elias EA, Elias RA, De Vries GP, Meijer AEFH (1982) Early and late changes in the metabolic pattern of the working myocardial fibres and Purkinje fibres of the human heart under ischaemic and inflammatory conditions: an enzyme histochemical study. Histochem J 14:445–459

Friedman PL, Fenoglio JJ, Wit AL (1975) Time course for reversal of electrophysiological and ultrastructural abnormalities in subendocardial Purkinje fibers surviving extensive myocardial infarction in dogs. Circ Res 36:127–144

Ganote CE (1983) Contraction band necrosis and irreversible myocardial injury. J Mol Cell Cardiol 15:67–73

Gebhard MM, Bretschneider HJ (1989) Myocardial protection. Curr Opinions Cardiol 4:803–806

Gebhard MM, Schnabel PA (1989) Limitierende Faktoren der Ischämietoleranz des Herzens. In: Grote J, Witzleb E (Hrsg) Durchblutungsregulation und Atemgaswechsel. Fischer, Stuttgart, pp 125–133

Gough WB, El-Sherif N (1989) The differential response to normal and ischaemic Purkinje fibres to clofilium, d-sotalol and bretylium. Cardiovasc Res 23:554–559

Hearse DJ (1988) The protection of the ischemic myocardium: surgical success v clinical failure. Prog Cardiovasc Dis 30:381–402

Henry CG, Lowry OH (1985) Enzymes and metabolites of glycogen metabolism in canine cardiac Purkinje fibers. Am J Physiol 245:H559–H605

Karch SB, Billingham ME (1985) Myocardial contraction bands revisited. Hum Pathol 17:9–13

Kawamura K (1961) Electron microscope studies on the cardiac conduction system of the dog. I. The Purkinje fibres. Jpn Circ J 25:594–616

Kehrer G, Blech M, Kallerhoff M, Langheinrich M, Bretschneider HJ (1989) Contribution of amino acids in protective solutions to postischemic functional recovery of canine kidneys. Res Exp Med 189:381–396

Kübler W, Schömig A, Senges J (1985) The conduction system and cardiac sympathetic system: metabolic aspects. J Am Cell Cardiol 5:157B–161B

Lurie KB, Argentieri TM, Sheldon J, Frame LH, Matschinsky FM (1987) Metabolism and electrophysiology in subendocardial Purkinje fibers after infarction. Am J Physiol 253:H662–H670

Mall G, Raumbausek M, Neumeister A, Kollmar S, Vetterlein F, Ritz E (1988) Myocardial interstitial fibrosis in experimental uremia. Implications for cardiac compliance. Kidney Int 33:804–811

Martinez-Palomo A, Alanis J, Benitez D (1970) Transitional cardiac cells of the conductive system of the dog heart. J Cell Biol 47:1–17

Michelson EL, Morganroth J, MacVaugh H (1979) Postoperative arrhythmias after coronary artery and cardiac valvular surgery detected by longterm electrocardiographic monitoring. Am Heart J 97:442–448

Mobley BA, Page E (1972) The surface area of sheep cardiac Purkinje fibres. J Physiol 220:547–563

Muir AR (1957) Observations on the fine structure of the Purkinje fibers in the ventricles of the sheep's heart. J Anat 91:251–263

Neely JR, Grotyohann LW (1984) Role of glycolytic products in damage to ischemic myocardium. Circ Res 55:816–824

Pape C, Kübler W, Smekal P von (1969) Morphometrie am Reizleitungssystem und Arbeitsmyocard des Kalbsherzens. Beitr Pathol Anat 140:23–37

Pattison CW, Dimitri WR, Williams DT (1988) Dysrhythmias following coronary artery surgery. A comparison between cold cardioplegic and intermittent ischaemic arrest (32° C) with the effect of right coronary endarterectomy. J Cardiovasc Surg 29:601–605

Pick EP (1924) Über das primum und ultimum moriens im Herzen. Klin Wochenschr 3:662–667

Pine MB, Caulfield JB, Bing OHL, Brooks WW, Abelmann WH (1979) Resistance of contracting myocardium to swelling with hypoxia and glycolytic blockade. Cardiovasc Res 13:215–224

Pomykaj T, Schnabel PA, Eins S, Gebhard MM, Richter J, Bretschneider HJ (1986) Ultrastruktur des Kapillarendothels während globaler Ischämie des Herzens. Verh Anat Ges 80:571–573

Ramsauer B, Schnabel PA, Schmiedl A, Bartels U, Gebhard MM, Richter J, Bretschneider HJ (1989) Schädigungsmuster von Pur-

kinje-Fasern und Arbeitsmyokard nach Kardioplegie und Ischämie. Verh Dtsch Ges Pathol 73:511

Reiser J, Anderson GJ (1981) Preferential sensitivity of the left canine Purkinje system to cardiac glycosides. Circ Res 49:1043–1054

Richter J, Schnabel PhA, Bretschneider HJ, Gebhard MM, Preusse CJ, Pomykaj T, Ulbricht LJ (1984) Ultrastrukturelle Veränderungen der kapillären Endstrombahn von wiederbelebten Herzen nach globaler Ischämie. Verh Anat Ges 78:383–386

Richter J, Schnabel PA, Pflug M, Gebhard MM, Bretschneider HJ (1986) Elektronenmikroskopische Untersuchungen an Purkinje-Fasern bei globaler Ischämie unter Myokardprotektion. Verh Anat Ges 80:567–570

Schiebler TH (1953) Herzstudie. I. Histochemische Untersuchung der Purkinje-Fasern von Säugern. Z Zellforsch 39:152–167

Schmiedl A, Schnabel PA, Mall G, Gebhard MM, Hunneman DH, Richter J, Bretschneider HJ (1990a) The surface to volume ratio of mitochondria, a suitable parameter for evaluating mitochondrial swelling. Correlations during the course of myocardial global ischaemia. Virchows Arch [A] 416:305–315

Schmiedl A, Schnabel PA, Haasis G, Mall G, Gebhard MM, Richter J, Bretschneider HJ (1990b) Influence of pretreatment on interstitial and intracellular space of canine left ventricular myocardium. Acta Anat 138:175–181

Schnabel PA, Gebhard MM, Pomykaj T, Schmiedl A, Preusse CJ, Richter J, Bretschneider HJ (1987a) Myocardial protection: left ventricular ultrastructure after different forms of cardiac arrest. Thorac Cardiovasc Surgeon 35:148–156

Schnabel PA, Schmiedl A, Gebhard MM, Pomykaj T, Richter J, Bretschneider HJ (1987b) Optimierung der Perfusionsfixierung des Herzens durch Kardioplegie. Verh Anat Ges 81:141–142

Schnabel PA, Gebhard MM, Richter J, Schmiedl A, Bretschneider HJ (1988a) Feinstruktur subendokardialer Purkinje-Fasern während und nach Ischämie: Einfluß unterschiedlicher kardioplegischer Lösungen. Z Herz-Thorax-Gefäßchir 2:54–61

Schnabel PA, Clavien H-J, Kehrer G, Ramsauer B, Schmiedl A, Gebhard MM, Richter J, Bretschneider HJ (1988b) Strukturelle Ischämietoleranz subendokardialer Purkinje-Fasern im Vergleich zum Arbeitsmyokard. Verh Dtsch Ges Pathol 72:581

Schnabel PA, Richter J, Gebhard MM, Mall G, Schmiedl A, Clavien H-J, Bretschneider HJ (1990a) Ultrastructural effects induced by global ischaemia on the AV node compared with the working myocardium: a qualitative and morphometric investigation on the canine heart. Virchows Arch [A] 416:317–328

Schnabel PA, Schmiedl A, Ramsauer B, Bartels U, Gebhard MM, Richter J, Bretschneider HJ (1990b) Occurrence and prevention of contraction bands in Purkinje fibres, transitional cells and working myocardium during global ischaemia. Virchows Arch [A] 417:463–471

Singal PK, Beamish RE, Dhalla NS (1983) Potential oxidative pathways of catecholamines in the formation of lipid peroxides and genesis of heart disease. Adv Exp Med Biol 161:391–401

Singer D, Bretschneider HJ (1990) Metabolic reduction in hypothermia: pathophysiological problems and natural examples. Parts 1 and 2. Thorac Cardiovasc Surgeon 38:205–219

Sommer JR, Dolber PC (1982) Cardiac muscle: ultrastructure of its cells and bundles. In: Paes de Carvalho A, Hoffmann BF, Liebermann M (eds) Normal and abnormal conduction in the heart. Futura, New York, pp 1–28

Stier A, Finch SAE, Schäfer H, Gebhard MM, Bretschneider HJ (1989) $^{31}$P-NMR spectroscopy of phosphate compartmentation during ischaemia in hearts protected by cardioplegic treatment. Free Radic Res Commun 7:293–300

Thornell L-E, Sjöstrom M, Andersson K-E (1976) The relationship between mechanical stress and myofibrillar organization in heart Purkinje fibres. J Mol Cell Cardiol 8:689–695

Tribulova N, Slezak J, Gabauer I, Styk J, Holec V (1988) Is the conductive system of the heart resistant to ischemia? Bratisl Lek Listy 89:647–654

Truex RC, Smythe MQ (1965) Comparative morphology of the cardiac conduction tissue in animals. Ann NY Acad Sci 127:19–33

Vander Heide RS, Angelo JP, Altschuld RA, Ganote CE (1986) Energy dependence of contraction band formation in perfused hearts and isolated adult myocytes. Am J Pathol 125:55–68

Vanderwee MA, Humphrey SM, Gavin JB, Armiger LC (1981) Changes in the contractile state, fine structure and metabolism of cardiac muscle cells during the development of rigor mortis. Virchows Arch [B] 35:159–167

Viragh S, Stoeckel ME, Porte A (1987) Light and electron microscopic structure of the cardiac Purkinje fibers – review. Physiol Bohemoslav 36:233–242

Weibel ER (1979) Stereological methods, vol 1. Academic Press, New York