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Original Article

CONTRIBUTIONS TO THE BIOPATHOLOGIC STUDY OF MYOCARDIUM BY REPEATED INTRAOPERATIVE MYOCARDIAL BIOPSY

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Abstract

The paper synthesizes a whole, long experience of the collective of authors of morphological evolution during open heart surgery, as in the literature have also reported data and methodology of the study. In 269 cases of acquired heart disease, especially cardiac malformations and various valvular heart disease. V. Candea performed myocardial biopsies, both before installing extra-body circulation (E.B.C), as a focal point of previous injuries and at every 5-6-10-15-17-20-30-35 sometimes 54 minutes after clipping the aorta to the involutive influence of E.B.C. and to the cardioplegic prefusions. Different morphological examinations were performed, especially using electonomicroscopy at Victor Babes Institute by the team led of dr. D. Laky. Under the influence of situation of "shock-controlled" ultrastructural changes – type stunning occur in the first minutes predominantly in the mitochondria, sarcoplasmatic reticulum, followed progressively by hypoxic injury at the vascular and cellular membranes. Evolution of mentioned lesions at the level of organites determines advance of the transition from limited reversibility of lesion, those 15-20 minutes, to the alterations appearance with irreversible evolution, from 30 minutes, with disorganization and lisis at sarcomers by lysosomal enzymes, edema peri and intrasarcoplasmatic, to apoptosis and necrosis. The beneficial aspect of treatment with phosphocreatin over. In cases of cardiac ischemia are given morphological aspects of myocardial hibernation as adaptive differentiate embryofetal, viable but with evolutionary potential in the absence of lesion of revascularization surgery. The paper pleads for the benefits of scientific and practical application of these methods by cardio surgeons as intraoperative investigation.

Keywords: *open heart interventions, intraoperative myocardial biopsy, biopathology*

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Rezumat

Lucrarea sintetizează în ansamblu, îndelungata experienţă a colectivelor autorilor privind evoluţia morfologică în cursul intervenţiilor chirurgicale pe cord deschis, întrucât în literatură nu s-au semnalat asemenea date şi metodologii de studiu. Pe 269 cazuri de cardiopatii dobândite, îndeosebi valuvulopatii şi variate malformaţii cardiace, V. Cândea a efectuat biopsii miocardice, atât înaintea instalării circulaţiei extracorporeale (C.E.C.), ca punct de referinţă al leziunilor anterioare şi la interval de 5-6-10-15-17-20-30-35 uneori 54 minute după clamparea aortei pentru a decela involutiv influenţa CEC şi a perfuziilor cardioplegice. Variatele examene morfologice, îndeosebi cele electronomicroscopice, au fost efectuate la Institutul "Victor Babeş" de colectivul condus de dr. D. Laky. Sub influenţa stării de "şoc controlat", modificări ultrastructurale de tipul siderării apar în primele minute predominând la nivelul mitocondriilor, reticulului sarcoplasmatic, urmate progresiv de leziuni hipoxice interesând sistemele de membrane vasculare şi celulare. Evoluţia leziunilor organitelor menţionate a activării leziunilor determină progresiv trecerea de la limita reversibilităţii lezionale, respectiv 15-20 minute, la apariţia alterărilor cu evoluţie ireversibilă, de la 30 min. cu dezorganizări şi lize ale sarcomerelor prin enzimele litice lizozomale, a edemului peri şi intrasarcoplasmatic, până la apoptoze şi necroze. Este redat aspectul benefic al terapiei cu fosfocreatină asupra structurilor cardiomiocitelor. În cazurile cu ischemii cardiace sunt redate aspecte morfologice ale hibernării miocardice ca diferenţieri embriofetale adaptative, viabile dar şi cu potenţial evolutiv lezional în absenţa revascularizării chirurgicale. Lucrarea pledează pentru beneficiile ştiinţifice si practice ale aplicarii de catre cardiochirurgi ale acestor metodologii de investigare intraoperatorii.

Cuvinte-cheie: *Intervenţii pe cord deschis, biopsii miocardice intraoperatorii, biopatologie*

Introduction

Wide range of laboratory investigations of the myocardium subjected to open heart surgery under extracorporeal circulation (E. B.C.) missing sequential biopsy studies on changes and evolution of intraoperative myocardial injury in the adoption of appropriate protective measures. Description of morphological cardiac lesions were performed only on single intraoperative myocardial biopsy by various authors.

This is done on open-heart surgery, clipping off the aortic bulb in terms of shock control (6) of stunning, named by various authors mentioned especially by Braunwald and Carp, defining contractile disturbances duration variable (in our case by clipping the aorta) and restore circulation (after declipping) persisting contractile disorders. During this period a number of series of metabolic injuries occur due to the changes at the level of organites, cytosol and extracellular matrix. These biochemical changes are necessary to know, considering the fact that due to high degree of congestive heart failure (CHF) in many patients, the prolonged duration of the operation, in some cases, and cardioprotective effect

infusions with various cardioplegic effect solutions (in continuous improvement) so still performing poorly, reversible changes may develop into lesions with irreversible trends. In the absence of such data in the literature, we considered useful to our experience, first performed in Romania, modern morphobiochemistry study, myocardial biopsy performed at specified time interval. In the period 1976-1981 were made in the Service of Cardiovascular Diseases, founded by V. Candea, at the Central Military Hospital, 242 various heart disease by the team led by V. Candea, whose myocardial biopsies were examined separately by Dr. D. Prunescu only electonomicroscopical at the Institute of Biology. Between 1982-1998 a total of 269 cases operated for valvular (mitral, aortic, mitral-aortic, tricuspidien sterile or effusion), or to correct heart malformations (DSIP, DSIV or both, Fallot disease), some associated with drainage anomalous vein, transposition of great vessels. (6) intraoperative repeated biopsies were studied by team led by Dr. Laky at V. Babes Institute performed numerous histopathological, histochemical, biochemical and electronomicroscopical studies (13).

In this study we included cases of pre, intra and postoperative myocardial protection with phosphocreatin (24). The results obtained conferred us the motivation to present the methodology of the study, adding to the usefulness of expanding knowledge on the evolution of all morphological and biochemical substrates in the context of these surgical interventions and non-identify electrocardiographic and sometimes disagreeing with the clinical assessment of a class of ICC in order to adapt appropriate therapeutic means. Stress that in any such case had operated physiological disturbances during and after the repeated biopsies in the context of most of the cardio surgeons adopt this methodology to study, many expressed their fear of complications or unmotivated considering morphological study in the context of range of laboratory investigations.

Material and Methods

Of the large number of operated heart disease, we selected for this study, 269 cases in which intraoperative biopsies were performed at the same intervals and morphological investigations remained unchanged. For interpretation of the results were taken into account by the ICC class (NYHA), most falling within the third class, the existence or not of HTP, mostly moderate, other associated diseases (especially diabetes, atherosclerosis, systemic hypertension) as the age (predominantly from childhood in malformations and in valvular disease sometimes exceeding 70 years with male predominant) The lot of study was formed by:

- Acquired heart disease and degenerative especially Valvular postrheumatic disease and, mitral, aortic, mitroaoric, tricuspidan less sterile and septic rarely subject valvulotomy and valvuloplastia.
- Different Congenital Heart Disease DSIA, DSIV, often combined, sometimes associated with anomalous venous drainage, Fallot Disease (Penthalogy, Tetralogy, rare Syndromes Pizzi-Lauby Trilogy) and Transposition of Great Vessels undergo surgical correction.

- Genetic Heart Disease consisting of a small number of Hypertrophic Heart Disease, Restrictive, Dilatative, topography corrected surgically.
- Atheromatous Ischemic Cardiomyopathy were performed biopsies only in atrial areas and diskinetic zone which bypass were performed by the collectives (Goleanu, Patrut, Mocanu) on 50 cases from their rich collection of cases, studies in which D. Laky performed immunohistochemical investigations.

Surgical interventions was performed under conditions of moderate hypothermia in open heart under E.C.B., cooled with ice cubes and serum physiological at 4 degrees Celsius cardioplegic infusion of crystalloid solutions, discontinuous and continued (Al. Popa), taking myocardial biopsies before clipping the aorta from the right auricle reflecting the morphological status of the heart and aorta after clipping and installation E.C.B., especially biopsies of papillary muscles, every 5, 6, 10, 15, 17, 20, 30, 35, sometimes 54 minute. The material collected was cut to perform the following techniques:

- Histological and histochemical on fragments fixed in 10% neutral formalin included in paraffin, performed the color HE, VG, PAS with amylase, Lie, alcian blue, Scherlach
- Histoenzimological, the frozen fragments at cryostat: techniques for SDH, citochrom-oxidants, LDH, acid and alkaline phosphatase, ATP-aze of membranes, LDH, catepsinaB, nonspecific esterase
- Biochemistry: enzyme marker, mitochondrial and lysosomal, determining values imunogramei K+, Mg++, Ca++, Na+ and water
- Electronomicroscopical transmission on myocardial tissue fixed in glutaraldehyde 2.5%.

Results and Discussions

The originally lesion pattern, built gradually in years, we studied on biopsies before clipping the aorta, set up criterion for assessing aortic lesions after clipping. Histological examinations revealed little information: vacuolation, deleting sarcoplasmatical structures, acidification of the cardiomiocytes cytoplasm (eosinophilia, glycogen changes, interstitial fibrosis, hypertrophy and hypercromation nuclear, thickening of arteriolar walls). We noticed histoenzimologic decreases to mitochondrial marker enzymes (citocromoxidase, SDH), leads to the biochemical and tissue fragments.

Electonomicroscopical exams revealed a range of progressive lesions whose emphasis we present below.

Literature data show normal structure organites and other components cardiomiocytes, injuries issues and their interpretation. Although, they revealed from the first minute in detail, the unfisiological effects of E.C.B., but and infusion cardioplegic solutions, suffering undergone cardiac "shock control" in stunning state.

Deterioration of circulation through the created shock by clipping the aorta, generating an infusion leading to inadequate tissue ischemia progresses to the extent that the shock and consecutive metabolic disorders. Thus in terms of metabolic cardiomiocytes from cell that consumes lactate for energy becomes a producing lactic cell. So an acidose cellular was installed with changes in cellular metabolism, decreased energy production, disturbance phenomena in the pump cell membranes, accumulation water and Na+ in the cell with edema cell subsequent. After clipping the aorta and installation of E.C.B. we found chronologically (6), progressive deterioration of subcellular organites and citostructural, at baseline were viable, adaptive of stunning-type (13, 14).

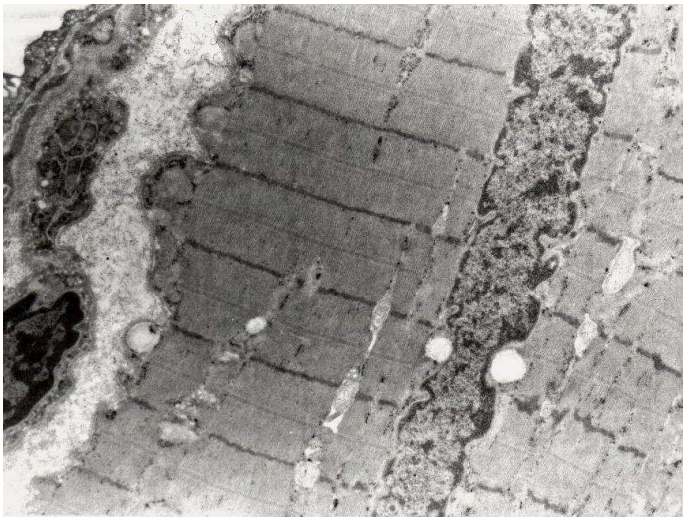


Figure 1 - Injury sarcolemal with vacuolisation, edema and intra perisarcoplasmatic, mitochondrial damage the Z lines (9500X), 13 minutes after clipping the aorta

Sarcolema presents early hypoxic alterations, ultrastructure illustrated vesicles of pinocitoza, and later by vacuolization subsarcolemal dehiscence, disturbances of diffusion hypoxia. Starting from the first minutes 2, 5, 6, 10, mitochondria (Figure 2) First organites affected appear (15). This is of particular importance because they occupy 25-30% of the entire cell, disposing it in regular rows between miofibrile and are its principal place of oxidative phosphorylation, electron transport, through oxidative phosphorylation plays a role in the formation of DNA and RNA at different levels (external membrane, internal, matrix) to store Ca++.

Their visual appearance varies, passing through a series of steps from the reversible to the irreversible, fragmentation and deletions of myofilamente, loss of granules to their disappearance, obtaining detailed images on mitochondrial suspensions (Figure 3). The appearance of electronodense deposits, represented by precipitates of Ca 2+, indicating the worst stage of lesions of the organites (Figure 4). Following the disturbances of physiological alterations of oxidative phosphorylation as reflected by decreasing the ratio P/Ca. Occurring disorders of permeability of membranes expressed through failure of Na+ and water influx of K +, Mg++, deficiencies Ca_2 transport.

Figure 3 - Irreversible damage to mitochondria with granular deposits with Ca. (4500X), 30 minutes after clipping the aorta

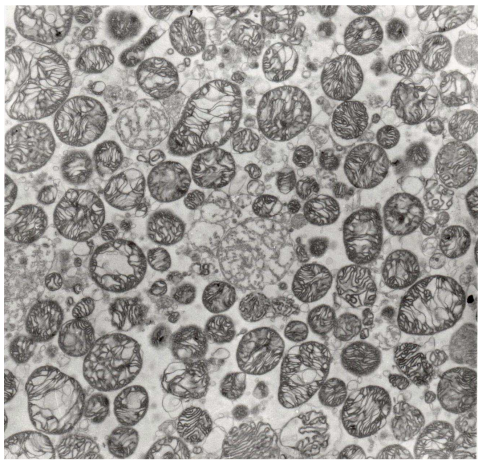


Figure 4 - Suspension mitochondrial: mitochondria vacuolation, avoid of content or being vacuolation (9500X) 30 min from clipping the aorta.

Sarcoplasmatic reticulum presents early postischemic alterations, which are characterized by expansion in the early stages that progress to the fragmentation of membranes and the appearance of plexiform areas in sarcoplasma (Figure 5). Lysosomes are organites that play an important role in the economy and the life cycle of cells especially in advanced stages.

Lysosomal alterations proliferated from perinuclear area (Figure 6) with various shapes and arrangements, issued by the disturbance of permeability of the membrane, protease, lipase, phospholipase, sulfatase, glicoronidaze, acid phosphatase, catepsine. They may alter the integrity of surrounding structures and function of cell metabolic changes incurred during the duration of clipping along with the advancement of the aorta.

Figure 5 - Cardiomiocit portion expansion of various sarcoplasmic reticulum, nucleus with cromatinian marginalisation, 20 minutes after aortic clipping (8000X).

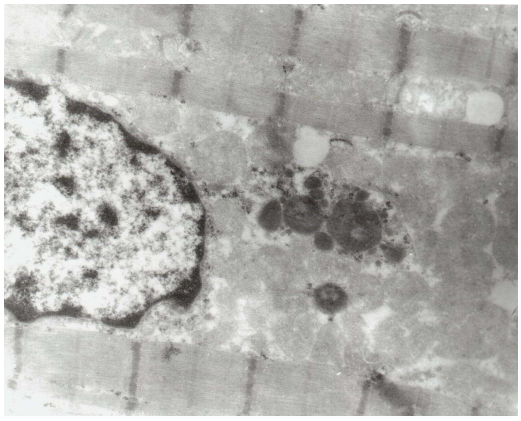


Figure 6 - Perinuclear lysosomes with extensive trend within sarcoplasmei with various injuries (11000X), 25 minutes after clipping the aorta.

All these stages of evolution from hyperplasia appear second lizozoms forming fagolisosoms (Figure 7). Finally, determine the enzymes released by the disintegration of structures leading to cell death concomitantly with the appearance of myelin bodies (Figure 8).

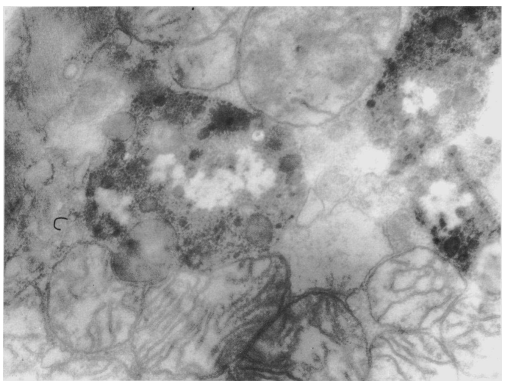


Figure 7 - Fagolizozomi crowded perinuclear (9500X) in 30 minutes

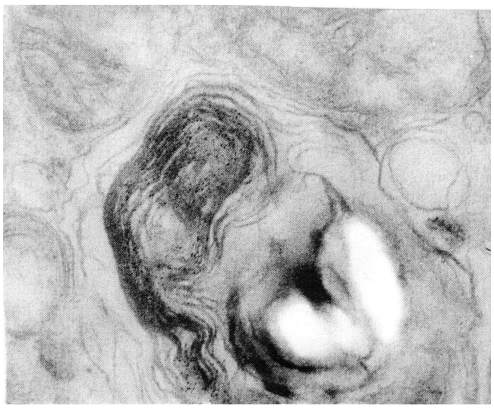


Figure 8 - Myelin body in the vecinity of mitochondria with rupture of growth and tendency to vacuolation (25000X)

Atrial natriuretic granules (16), become visible in growing numbers in conditions of hypoxia (17). The repeated biopsies, we have met and in the ventricles (18). The aspect is spherical with electron dens content of 2.75 (Figure 9) located near Golgi apparatus and that secrete hormones and peptides. Through its activity give endocrine feature to the heart (18). Fluid balance influences Na-H_2O issued consecutive ion pump damage (17, 18). They are characterized by changes of the inflow intracellular hidroionical Na+, water and Ca 2+ and the efflux of K+ and Mg 2+. Resting potential decreases hence the decrease of excitability cellular. The number and their extension increases with worsening heart failure.

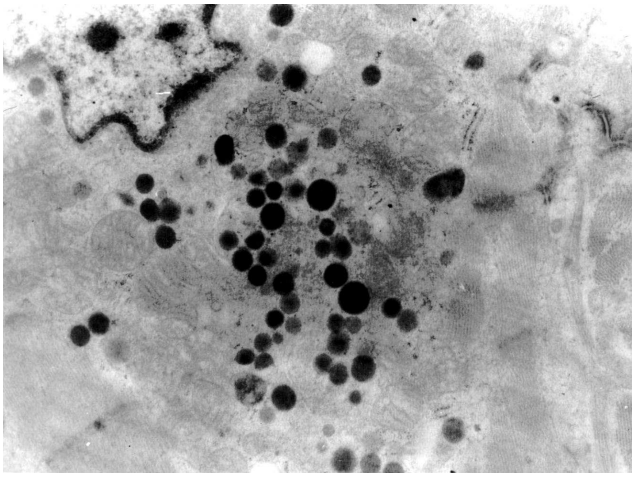


Figure 9 - Proliferated natriuretic granules in the vicinity of the nucleus inside sarcoplasm

Myofilaments and discs gradually suffers interlacing dissociation and disintegration mainly due to edema intrasarcoplasmatic, tapes and discs is fragment insertion, the intherior sarcomer structure, nuclear changes occur in advanced stages and, ultrastructural, they are showed by marginalization heterocromatin and by the appearance of dense chromatin.

Extracellular matrix causing suffering progressive morphobiochemical alterations, finally lead to myocardosclerosis and increased heart failure (31), capillaries present changes of basement membrane, with vesicles of pinocitosis increasingly numerous and endothelial cells appear turgid and festoned.

Ultrastructural aspects vary from one microscopic field to another even within a sarcomer, that the labeling stage lesion requiring multiple sections. The accuracy of these changes electronomicroscopic is certified by esperiments results which simulates various forms of myocardial ischemia observed by team led by Dr. Laky for a decade, and described in his monograph (14). Both valvulopaties (19, 20) and malformations (21) painting ultrastructural lesion was nonspecific and intercept compared with the previous state of the patient especially ICC class (NYHA), a pre-existing hypoxia, alterations observed in the previous biopsy experience and caution required by the Examiner. In this way, were determined adaptive changes within sideration process, reversible early lesions from those, that are at the limit of reversibility after 15-20 minutes morphofunctionaly viable and the lesions that evolved after 20-30 minutes to irreversible status of apoptosis and necrosis. Following the dynamic changes in myocardial cardioplegic infusion solutions, we found in the majority of cases, their efficiency in miocardoprotection (14), lesions, with few exceptions, not exceeding reversibility limits of lesions.

Alexandru Popa has devoted his doctoral thesis to efficiency continuous infusion of cold cardioplegic hyperkalaemic solutions (22). Inauspicious results, demonstrated electronomicroscopicaly, were noticed in cases of ICC class IV (NYHA) in endocarditis with septic embolism (22, 23) and in some patients, in whom the time of surgical operators have exceeded 45 minutes, such as, in double or triple valves prosthetic process or complex malformations.

In these patients we found hyperplasias and lysosomal activation, frequent phagolisosoms, increased tendency to swelling of mitochondries, the interstitial and intrasarcoplasmatic edema. Myofiaments appear dissociated from edema, with changed Z band with appearance of structures called "Z band material", dilatation, with plexiform images of the sarcoplasmatic reticulum, increasing numbers of natriuretic granules, subsarcolemal vacuolisation.

Capillaries present vesicles of pinocitosis common in basement membranes, and in the final stages of lysis, appear myelinic bodies, mitochondrial swelling and progressive sarcoplasmatic lisis with nuclear picnosis. Biochemical exams were consistent, signaling decreases in mitochondrial enzymes and increases in the those lysosomal. From histoenzimological point of wiew, values of acid phosphatase, catepsin B (lysosomal enzymes), as well as LDL, were consistent with the severity of lesion images. A particular aspect we studied was that of miocardoprotection pre, intra and postoperative with fosfocreatina (Neoton) (24). For accurate results were studied two groups of patients operated for

valvulopaties respectively untreated and treated. In these groups, biopsies and investigation techniques were the same as in previous cases. In cases where myocardial biopsies from patients treated with Neoton them, the picture appears with lesions morphologically reversible (Figure 10), compared with untreated group presents severe lesions (hyperplasias of fagolizozomi, sarcomeres with advanced disintegration with mitochondrial damage and edema) (Figure 11).

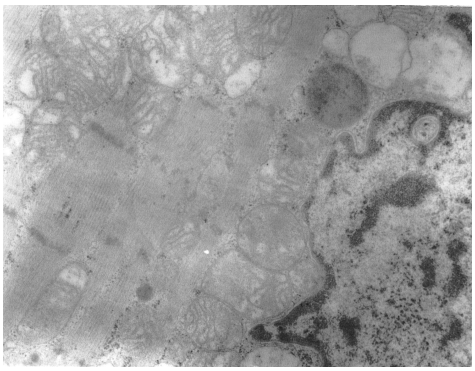


Figure 10 - Miocardium after pre and intraoperative treatment with phfosphocreatin- injuries lightweight, reversible, with moderate dilation of the reticulum sarcoplasmatic, light mitochondrial changes, disruptions of the Z lines (12000X), 20 minutes after clipping the aorta

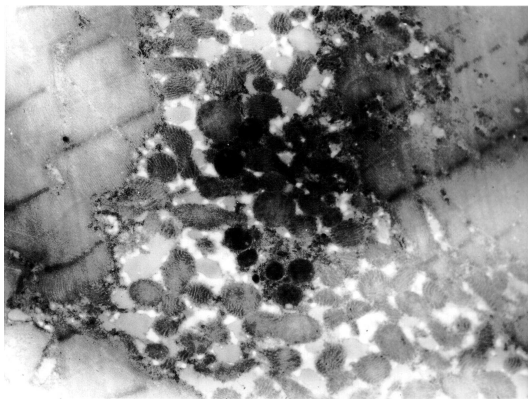


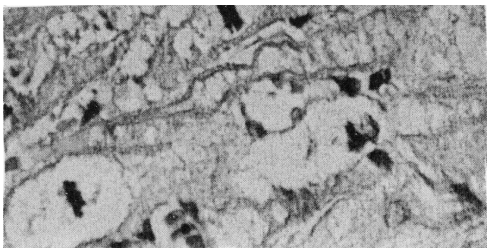
Figure 11 - Myocardium in a patient with untreated fosfocreatin (Neoton) presents: fagolizozom severe alteration of mitochondries and deleting sarcomeres structures (12000x), 20 minutes after declampation

So, the apparent tendency of glycogen recovery and sarcoplasm acidification and myocitic edema are much lower. Histoenzimological appearance of ATP-asis membrane, the mitochondrial enzyme and LDH remain in limits before installing CEC. Lysosomal enzyme activity is reduced, so the aortic clipping the changes appear smaller.

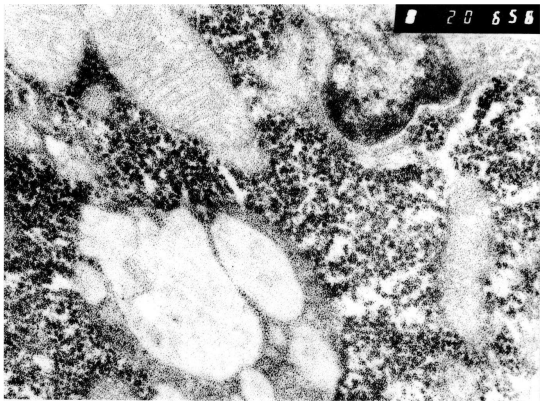
Electronomicroscopic examination (Figure12) also observed a better preservation of cellular organits, storage non-fragmented miofilamentes [12a], Z bands and disseminated intrasarcoplasmatic glycogen granules [12b], and between miofilamentes, mitochondries with membranes and crests preserved. Vacuolo-lysosomal system is reduced by untreated group, suggesting a slow phenomenon cytolysis [12c].

Bazal membran of endothelial cells present an increased stability, causing perivascular edema reduction, inter and intramyocitar.

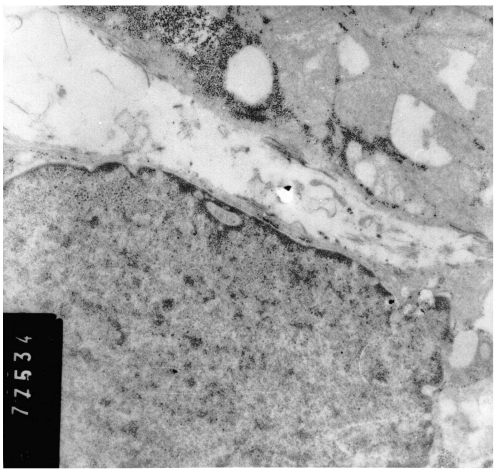
1. Restoration of spontaneous heart rate.
2. Reducing energy needs due defibrilation.



12a - myofibrilar severe depletion leading to waved arrangement of sarcomers, with a tendency to vacuolisation (4500X)



12b - glycogen granules heavily congested in sarcoplasm (4500X)



12c - The core of pulverized chromatin, deletions of sarcomere structures (4500X)

Figure 12 - Electronomicroscopic imagies of myocardium in patients with chronic myocardial ischemia, with biopsies harvested during by-pass aortocoronarian site.

These findings have been demonstrated both experimentally and clinically. In ischemia, the myocardial energy reserves (ATP, CP), are fast consumers and glicolysis stops at gliceraldehidphosphat, step leading to loss of oxidation phase of cellular NADH-, causing acidification of cytoplasm.

Neoton, is a source necessary to prevent oxidative phosphorylation stop and avoid metabolizing phospholipids in membranes, resulting sarcomeres stabilization and membranes capillary. It is used to restore mitochondrial ATP reserve, sarcolemal and myofibrilar. Neoton prevent irreversible changes in reperfusion by maintaining tissue pH, blocking sarcoplasm acidification because

accumulation from lactate and alanine, results in high consumption of glucose and lactic acid, preventing injuries.

In cases with varying degrees of chronic ateromatos painful ischemia subject revascularization by aortocoronarien by-passuri, were performed electronomicroscopic examinations on biopsies from ear before practiced graft (50 cases-Dr. Laky), and during bypass from dikinetica area (Dr. Patrut, Goleanu and Mocanu), showing contraction disturbances by adaptive changes, states of stunning, hibernation and various injuries viable reversible, with a tendency toward irreversibility, characterized by apoptosis and necrosis.

Siderotic changes have show viable lesions, especially of mitochondria and reticulum sarcoplasmatic, which were in a study and can pass to hibernate and evolving lesions. In these chronic ischemic heart disease, I met a state of hibernation, the chronic disorder, consecutive to moderate chronic ischemia severity, in which cardiomiocytes remain viable, their contractile function is impaired, can be restored by surgical reperfusion, preventing passage of moderate hypoxic lesion.

Emphasize the fact that myocardial hibernation isnot a lesion state but adaptive, remodeling, the self-protection and adaptation to infusion.

Can be identified morphologically and histologically by the appearance of pale cardiomyocites by lysis of miofilaments and persistence of glycogen.

Electronomicroscopic, along with miofilaments lysis (substrate of diminishing contractility evidentiated clinical mainly by stress on dopamine and nuclear medicine techniques), I met the dense granules of glycogen, nucleols with various aspects prevailing in chromatin, pleading for embryo differentiation, state in which contractions are reduced compared to postnatal cardiac activity.

These data were certainly evidenced by immunohistochemical examinations, prezence of actinic smooth muscle, modification of contractile proteins that titina and cardiotonin and persistent sarcoplasma growth factors, changes in some quantities of calcium and the absence of fibronectin in citosol, the nuclear lamininei H and C. In the basic data, add the rare observation of tubular T system, as reticulum sarcoplasmatic, absence of mitochondrial swelling and lysosomal activation as well as the extracellular matrix changes. These processes consist in changes of mucopolizaharidic parts, cell growth, that of macrophages, fibroblasts, miofibroblastes, capillary density and alterations of their endothelial function. In chronic hibernation increase mainly structural proteins collagen type I and III.

Diagnosis of state hibernation by means of physiological and nuclear medicine techniques, requires emergency surgical revascularization of these diskinetic areas, there can return to normal, but also to prevent their growth by chronic hypoperfusion to progressive ischemic lesions, reaching on apoptosis, necrosis and substitutions collagen. Revascularization should be noted that all areas ischemic oxygen free radicals have bad efects, being necessary to combat their drug means.

Conclusions

Intraoperative myocardial biopsy repeated by our team during open heart surgery sub CEC on a large number of cases, within two decades and examined morphologically complex, especially electronomicroscopic, they evidentiated the evolution of lesions for operations, enriching data literature.

This study, advocates for intraoperative cardioprotection continuous such as cardioplegice infusion, administration of drug solutions in the prevention of postoperative complications.

Intraoperative myocardial biopsies at different intervals of surgery during bypass aorto-coronary sites in areas diskinetice, have not shown in our studies or postoperative intra accidents, which is why we recommend Cardiochirurgia application is useful.

Evolution of pre-and intraoperative myocardial injury, surgestive compensated stages, to avoid irreversible damage.

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