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OBSERVATIONS OF APOPTOSIS IN MYOCARDIUM IN SOME CARDIOPATHIES ON INTRAOPERATIVE AND EXPERIMENTAL MATERIAL

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Abstract. *With a view of apoptosis signification in progression of cardiopathies, our studies have been used morphological investigations on specimens from human intraoperatory biopsies and experimental material. It has been used histologically, histoenzimologically, electronomicroscopically and in some cases molecular biologically investigations. The differentiation between necrosis and apoptosis were difficult, these being isolated thought decreasing cellular volume, cytoplasm densification, slow lose of organelles affection, except mitochondria and nucleus. The certification was due by molecular biology techniques applied TUNEL-DIG and propidium iodide methods which distinguished no reversible lesions of DIVA from apoptotic bodies in myocardial infarcts and myocardium sclerosis border lines, in chronic ischemia areas of hibernation, myocardial hypertrophies, in advanced heart failure. On experimental models similar to angina pectoris(ischemic and reperfusion alterations) and chronic ischemia through partial coronary obstructions, it had appeared apoptotic images like those which are find in human pathology. There are mentioned genetic mechanisms involved in apoptosis and stimulating factors that perturb its, determined the progression of heart failure, and the benefic therapy that would have applied.*

Keywords: apoptosis, myocardium pathogenesis, cardiopathies, experimental researches of apoptosis, long term therapeutic prospect.

1. Introduction

In the last decades, the studies about apoptosis, especialially concerning molecular mechanisms, have progressive increased. In the frameword of these dynamic studies, we shall try to present our experience about apoptosis in cardiopathies because in literature there is a few data about this subject. In this sense we have see again the extensive material which we have gathered in almost 3 decade [1, 2, 3, 4, 5, 6, 7, 8, 9, 10] and have processed the recent harvest references [11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26]. We have compared the results of our researches with those who are appeared in literature and we do some reflection about the pathogenesis of these lesions.

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2. Material and methods

For this study we have used:
Biopsia material – intraoperatory harvested over 250 cases from Center of Cardiovascular Disease of the Army (Cândea, Țintoiu, Pătrauț, Goleanu, Mocanu et al.) and from Institute of Cardiovascular Diseases "Prof. Dr. C.C. Iliescu" (Cândea, Făgărășanu et al.). The biopsies are harvested from different areas of myocardium at various time intervals, in the framework of the same case with a view to praise the hypoxical myocardium changes consecutive extracorporeal circulation (C.E.C.) with continuous and discontinuous perfusions with cardioplegic hiperpotasemia cold solutions.

– postatherisclerotic ischemia cardiopathies,
– congenital cardiac malformations.

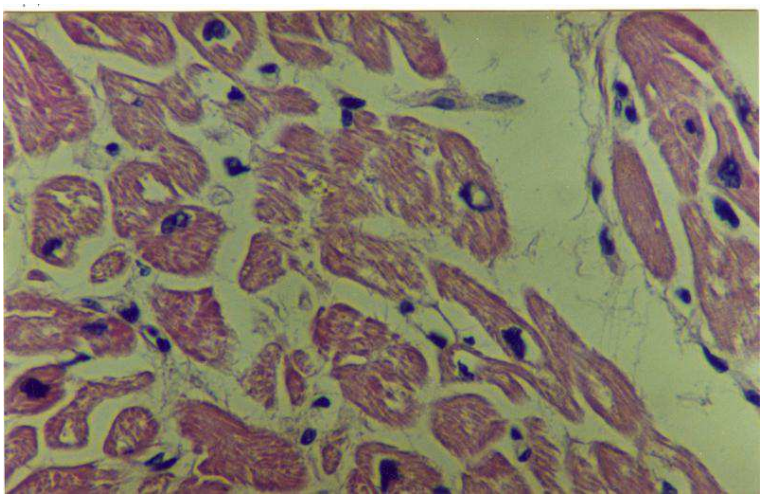

Figure 1. Myocard, chronic ischemic cardiomyopathie intraoperatory
Biopsie (I.G.).N.E. staing 400x cardiomiomyocites with apoptotic and necroting appearance;

Experimental matheried harvested from 117 dog, in years interval 1971 - 1980 from "Victor Babes". Institute by a group leaded towards D. Laky and N.M. Constantinescu ("Carol Davila" Institute for Medicine and Pharmacy). At these animals it was effected acute ischemia from 5 at 60 minute [3, 4]; at other dogs – the partial obstruction of left circumflex coronary branch or left anterior descending during 10 days (5, for following the acute myocardium infarct evolution; the transitory ischemia [6, 8], coronary stenosis follow on recirculation's, while at others animals after the same proceeding incomplete bundle of that coronary brands during 60 days. At all cases the specimens are fixed in neutral formaldehyde 10% and are embedded in paraffin, they were sectioned at 5 microns and were staining utilizing the following histological

technique: Haematoxilin - Eoxin, Van Giexin, Lie, PAS, PAS+Amilase, PAS-Aleian, Gomori.
For histoenzymological investigation the fragment were frozen sectioned at cryostat (-20°C) and were affected the techniques for SDN, citocrom oxidase, NADM - C - Citocrom-C reductase , membrane-ATP-ase, acide phosphatase, LDH.
For imunohistochemical techniques on human material, the sections were affected from paraffin block, were investigated for evidence of smooth muscle actin, VIII factor and methods of macrophages.
For electronomicroscopy studies, the fragments of 1 mm were fixated in glutaraldehide solution H%, processed for embedding in 812 semifine and fine sections, were examinated and photographed at electonomycroscopies Optan and Jeol.
In some causes we are affected research of molecular biology on frozen myocardium tissue and paraffin sections, respective TUNEL-DIG and propidium iodide for visualize the state of nuclear DNA.
On experimental material we have done in an addition, biochemical technique
(Sm. Constantinescu, Gab. Filipescu) on mitochondrial suspension with a view to spotting the marker enzymes [3], while on lisosomal suspensions, acid phosphotase and beta-glucuromidaze. In some experimental cases it was affected on myocardia tissue specimens establishing of water amount and ionograme (particularly of K, Wa and we have measured the ATP quartiby [7].

3. Results

We have examinating and reexaminating the older histological images that are specific apoptosis state. We are found histological descriptions that are specific feature of apoptotic state. We are found that the detailed histological descriptions that are done even before of elaboration by Kerr in 1972 [23] the apoptosis term and subsequent amplification have corresponding entirely with specific features who are précised up to date through microscopic methods inclusive some morphological stage of this evolution.
The fact that we are not mentioned the apoptosis term were clone of these still unelaborated nomination that are difficult established, even it is missing from reputate monogephies of contain know authors [24, 25]. Only in very obvious cases we have utilized the term of irreversible lesion of apoptotic type; we have mentioned particularly after electronmicroscopical exams the term that “limit of morphofunctional reversibility” that is equivalent today with apoptotic states or reversible lesions (especially in sideration and hibernation states) [1, 2]. The great difficulties to specify the cellular viability or death on usuales staines because of intricated images who included between they areas the fibrosis focuses. In this sense we are become precise after the amplification of our investigations and

study of recent literature the busis features of apoptosis and necrosis with a view to their differentiation [8, 11, 13, 14, 23, 24]. Thus in apoptosis cases the nuclear lesions early appeared their evolution is able to watch electronmicroscopicaly in sense that in early studies the nuclear cromathin is perinuclear condensed then undergoes progressive fragmentations until disintegration of diereses portions of nucleus. The cells apoptotic affected appeared isolated, in small groups, separated from the neighboring cells. The cell volume decrease until half, the cytoplasm density consecutive of dehydration the organelles appear in majority unmodified except the mitochondria which are early damaged. The sarcolemal lesions appeared to late and cytoplasm disintegration as well as of nucleus and made up the apoptotic bodies with acidified cytoplasm. All around, we aren't found the inflammatory reaction, we are found only macrophages and nuclear changes, show apoptotic changes, the nucleuses are hyper chromatics, irregulars here and the clivates and doubles and poliploids sometimes (**Figuere 2, 3, 4**).

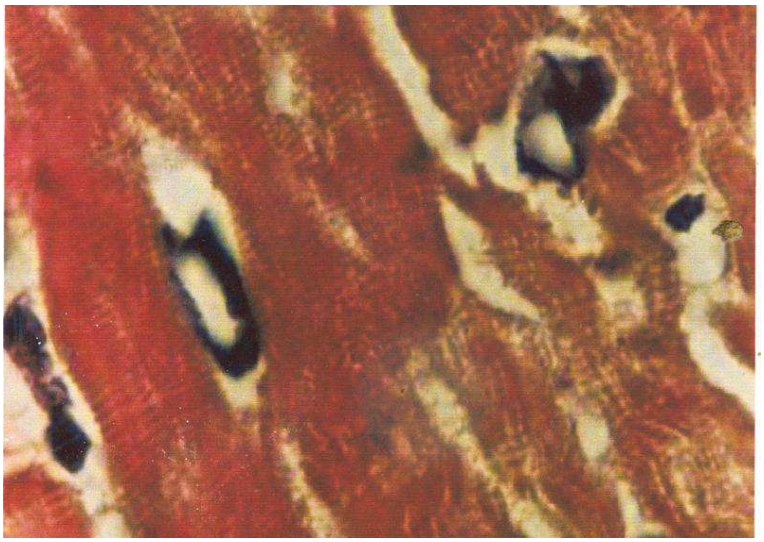

Figure 2. Myocard, chronic valvular disease, intraoperatory biopsie; Lie staining, H.E. staining, immersion;Hypertrophic cardiomyocites with large hipercro matic nuclei with apoptotic appears;

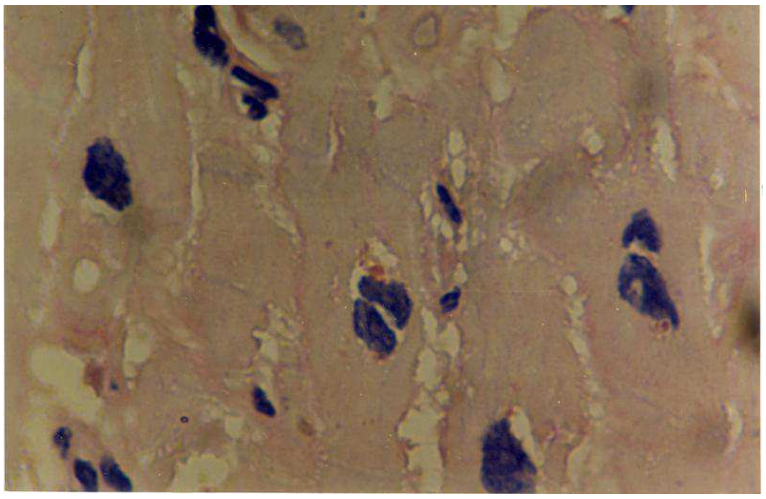

Figure 3. Myocard intraoperatory with malformations (SDA, SDIV), PAS-amilaze staing 400x myocardial cells with double nuclei; Hiperchromatic and with vascuolization

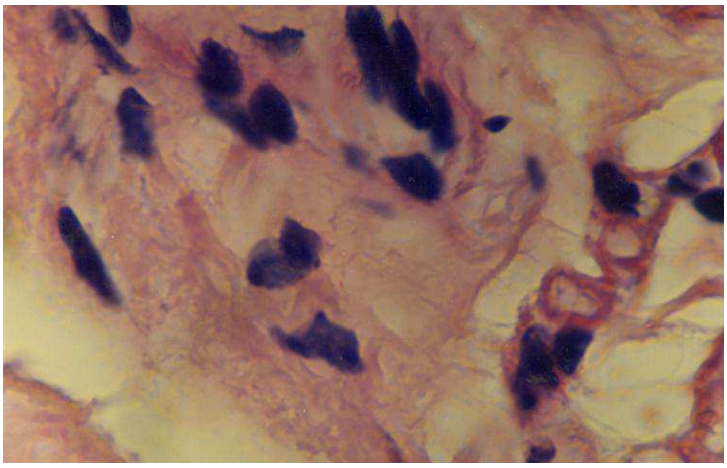

Figure 4. Myocard intraoperative with malformations, PAS - amilaze staining, imersin; Other image with nucleary modifications to poliploide;

The process spended with energy consumption, is a mechanism of cellular programmed death [17], named of some authors as well as “cell suide” [26]. In the end, the apoptotic bodies are fagocitate from macrophages or the neighboring cells and they are digested of lysosemal enzymes.

The necrosis are late nuclear lesions who are gradually installed through irregularly condensed and then homogenized nuclear chromatin. The organelles undergoes to severely lesions consecutive their early evolutive modification while the sarcolema had membranes lesions. The mitochondria appeared swelling with

disruption and disintegration of crests. The necrosis process doesn't required energy consumption. Both lesions are unspecific for some organ gived, they presented and of myocardial level the some images. The new techniqs of immunohistochemia show embryofetalis dedifferentiations of some cardiomyocites throng presence of smooth muscular actine in their sarcoplasma and of macrophages in their neighboring [1, 26]. The stainings Lie and aleian-blue, show acidification of sarcoplasmatic medium who is favorable for apoptotic processes. The Gomori staining as well as ultrastructure praised the presence of fibrosis focars in zones with cardiomagocitare depletions consecutive above mentioned processes. The capillaries presented endothelium turgesence this aspect is augmented ultrastructuraly detailed appeared at capillary basal membrane level.

Electronomicroscopicaly we can establish because of disseminated character of lesions, organelle's modifications, rather of nucleus, chromatin appeared irregularly dispersed in clusters with tendency to vacuolization [5, 6, 7]. The appearance of lysosomes and phagolysosomes indicate the possibility of evolution to necrosis of these [4, 5] alternative cellular changes.

The osetiacellular matrix edema, rather in condition of organization, acidification and hyaline, the fibrins transformation through the presence of collagen synthesized by fibroblasts is an cellular worsening index-lesion [21, 22].

Apoptotic changes who evaluated to necrosis can appeared through physiological stimulus rather in older age but consecutive the emphasis of light preexistent lesions through the appearance of some hipoxical, toxical and mechanical damage who are quickened thus the installation of apoptosis and then, of necrosis [3, 4, 7, 9, 10, 15].

Depending on severity of harmful agent, the lesions can evaluate to necrosis, to apoptosis (**figure 3, 4**) or structural restoration. Thus, in acute myocardium infarcts, the central area is always necrotic but in peripherical areas are intricated myocites with manifest tendency of necrosis with the other [4, 5, 6, 7, 8, 19] which often presented the waved aspects while electronomicroscopicaly features of hibernation [10, 21, 26] with agglomeration of glycogen granules, endoplasmic reticulum changes, fine and dispersed nuclear chromatin who can evaluated to apoptosis or structural restoration rather in conditions of an suitable treatment that are limited the extension of infarct [19]. Such heterogeneous cardiomyocitares changes we have been met in the neighboring of ventricular anevrism consecutive on sclerohialinisation of myocardium infarcts [10].

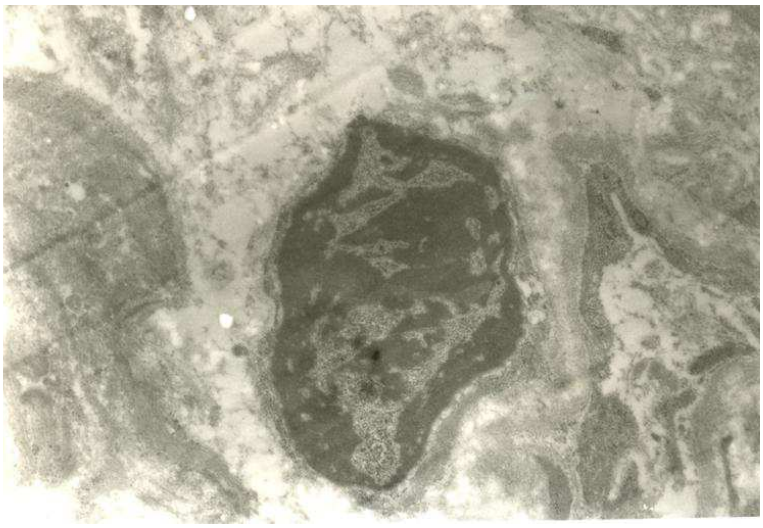

Figure 5. Myocardium: intraoperative biopsy E.M.9100x: disintegration of nuclear structures;

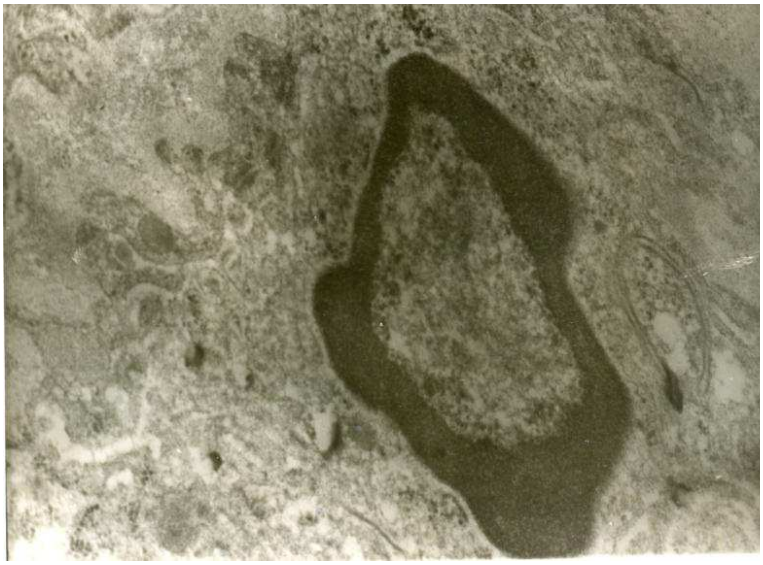

Figure 6 .Myocardium: intraoperative biopsy from left myocardium, chronic valvular disease (SDIV, SDA); Cardiomyocite with nuclear vacuolization E.M.X9100;

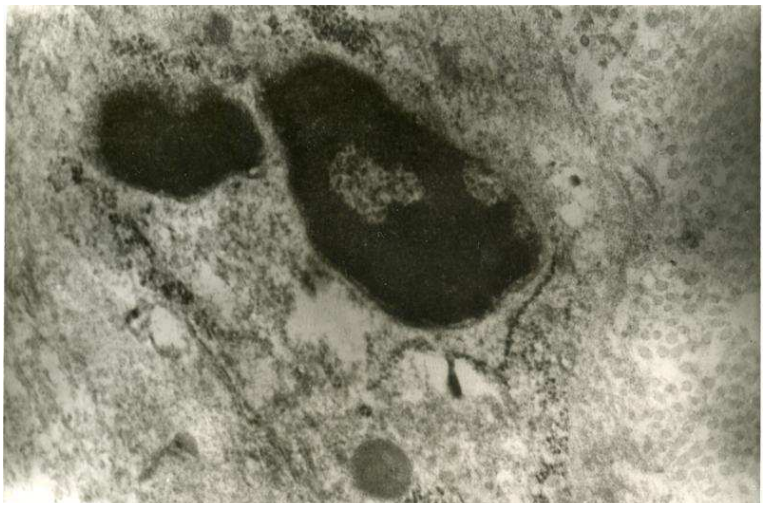

Figure 7. Myocardium with chronic valvulopathie, severe cardiac failure E.M.9100x;
Hiperchromatic nucleus with central structural disintegration, nuclear body with hyperchromas;

In zones with consecutive myocardial ischemia of advanced coronarian atherosclerosis, they are such intricates lesions often of hibernation type with nuclear reversible apoptosis through the vascularisation of these areas through aortocoronarian by passes [22].

In valmeopathies [2], rather mitroaortical, we have been met apoptosis, as well as atrial level after fibrillations and ventricular level because the hypertrophy of cardiomyocites with formation of news sarcomeres and multiplication of myofibrilar contractile apparatus. In these situations because of great size of nucleus and of chromatinian and nucleolar volume is sometime difficult to specify the moment of transitions toward apoptosis. That take place rather in the case when arised sarcoplasmatical micro vacuoles and acidification of sarcoplasma. Their energic resources exhaustion consecutive of constraint trawell asa well as the hypoxic state in which they will be because the inadegvatic development of neighboring capillary network size. Similar aspects we have been met also in cardiac malformations, the lesions are unspecific [9]. At histological routine methods appeared nuclear changes who indicated apoptotical states; the cromathin condension under the nucleolema, the appearance of vacuolisations in the middle of the nucleus, their intended outline, tendency to clivatien and their session, appeared cells with 2 nuclei.

In our casuistry we have been met displasia we haven`t been met the right ventricular arritmogenic right ventricular dysplasia in which the literature data show an increased number of apoptosis [16].

Immunohistochemicaly, we have been establishing [10] existence of smooth muscle actin show their embriofetal dedifferentiation and they have keeping the morphological features of their viability.
The same processes we have been observing in case of hipertrophias of certain zones in the framework of inheritance heart malformations through similar mechanism [9].
We can certainly précised the apoptotic states by molecular-biology investigations, that can through the visualizations, the degree of DNA-chain lesson, utilizing TUNEL-DIG technique (**figure 8**) and propidium iodatum rather in the cases of treatment of section with triposine (**figure 6**) that have permit the evidention of diverse states of apoptosis from those reversible to appearance of apoptotic bodies.

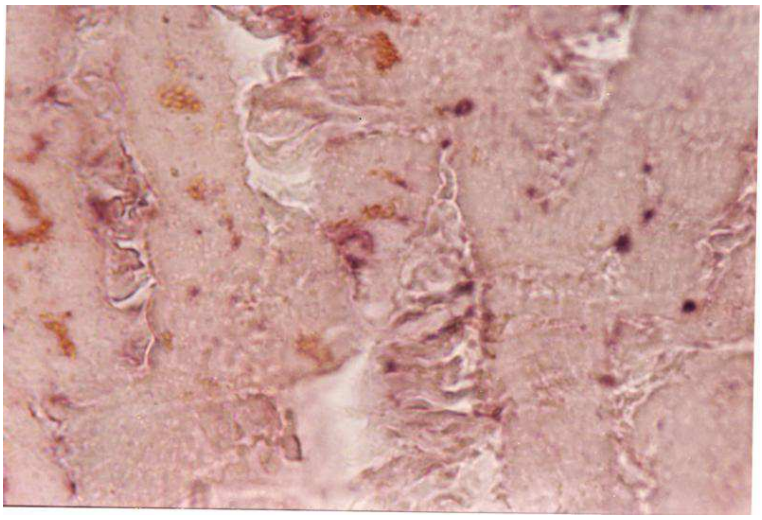

Figure 8. Myocardium, aortic valvular stenozis. TUNEL-DIG molecular biological staining, disintegration of nuclear chromatic x400;

On experimental material we were met in some models which we were effect the images alike with those observed on human matheria. The conception of these models is similar of certain human cardiac affections above-mentioned.

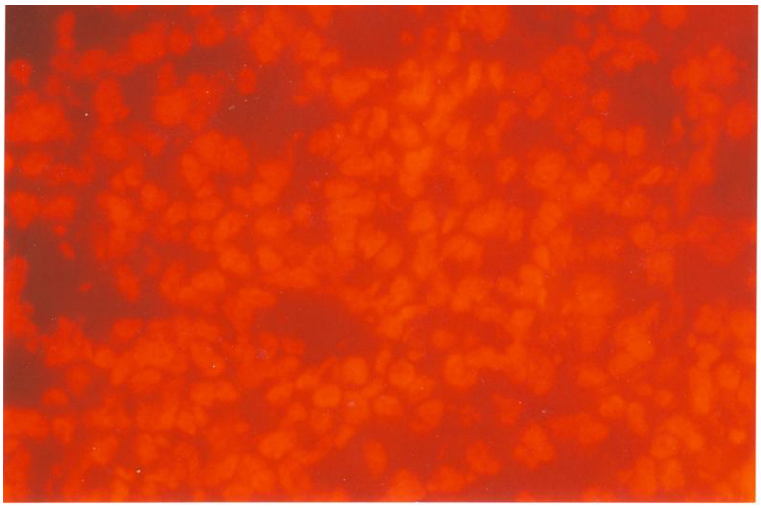

Figure 9. The same. Propidium iodatum, molecular biological staining on view apoptotic bodies.

4. Discussions

Our researches as well as the latest which are arising in literature
[2, 5, 10, 14, 15, 17, 22, 24, 25, 26] show the two patterns of cellular death of myocardium level: - necrosis-considered in past like the singular modality of cellular death, apoptosis-after recent researches.
Our researches are doing to differentiate the apoptotical lesions from necrosis although the both lesions have the same denominator: the lesion of nuclear DNA.
Apoptosis is a physiological state of cellular programming death [12, 13] named by certain auto cell-suicide view of maintain of quantitative homeostasia in rapport of the cellular cycle evolution, the age, the remarking and the differentiation of respective structures through energy consumption.
Because that, the apoptosis have been presenting small organelles lesions (rather mitochondria`s) cell volume decease with cytosol condension because of dehydrations, the nuclear lesions appear early while these of sarcolema arise late. They appear isolated from the neighborings cells without the inflammatory reaction all around. The length of apoptosis evolution is small but the number of these lesions and their evolution to necrosis can be accelerated through nociceptives stimulus's [10, 11, 12, 13, 14, 17, 26].
Necrosis have been appearing consecutive of the damage in cells groups because of some strongs or prolonged noxes have increased cell`s volume through sarcoplasmatical edema, early sarcolema lesions, severe nuclear damages but

belated appeared through prolonged evolution; the mitochondrial lesions appeared early and are intenses (swelling) and don't required energy consumption.
We considered necessary to present in brief the mechanisms of apoptosis [10, 12, 17, 27]. These are multiple mechanisms complexes, started on inducing factor and continued on the genetical, biochemical changes witch can goes to cell death. Among the determination role of cellular cycle [17], the restoration capacity of DNA; the free intercellular Ca++ ions concentrations RNA or certain protein synthesis or degradation modifications of phosphorilution and dephosphorilution; the oxidatives cell-mechanisms with generated of free radicals (reactive oxygen).
They have been coinciding by early morphological changes, chromatin condensation undergoes to clivatien of internucleosomales DNA which have been evidently through agar-gel electrophoresis [26].
The selective lose of water an ions with cell volume decrease on half in the earlier states of apoptosis, will contribute subsequently to fragmentation as well as cytoplasm to the nucleuses and apoptotic bodies-formation. The increased level of catabolic ionized Ca++ can activate the enzymes like transglutaminase and precocious induced apoptosis. That in why, the cardioplegic solutions [10, 22] antioxidant drugs can block the basic lesion of DNA and, consecutive the apoptosis installation. An important role have the cistein - proteases.
Among these we mention: the interleukine conversion enzyme (ICE) and especially the causes [20].
The apoptosis activation are effectuate through an series of factors which accentuate the histological and phisiologicical changes preexisting like the stimulation of myocitare hiperthrophy, interstitial fibrosis, vasoconstrictors like the others umoral factors: norepinephrine, angiotensine II, inflammatory cytokines, nitric oxide, GMPc - atrium, nathriuretic factor which induced apoptosis through still not clear up mechanism.
The genetic determinism of apoptosis have been observed initially at unverthebrates, they existed the apoptotic gene like ced-3 and ced-4 between ced-9 gene which have seen protecting the cells by programmed family bcl-2 which inhibited the cell death [27]. This gene can be inactivated by the genes bax and bel-x through combination with bil - 2 [6, 7, 20].
The intervention of suppressor gene P53 can suprimate the bel-2 activity; the gen P53 have been determinating to stopped of cell cycle in G1 state permitted the lesions restoration or determined the apoptosis. The gene c - myc can induce also, the apoptosis.
In this processes have been intervening and the Max-gen [26, 27]. The mitochondrias represent the decision factors about the cell-death type. Through the cytochrome - c releasing apoptosis are been induced [17]. Among the modulators of apoptosis, a decisive role has the caspases from cistein protheasis

family which, as well as ced - 3 actionated like effector of this processes. The caspasis can be inhibit by be - 2 and bel-x1 homolog ced - 9.
Determined the irreversible proteolysis would be beneficially by drug. The treatment with peptidic inhibitors of caspases which reduced the cerebral infarction dimensions with 50%. The limiting framework of this paper haven't been permitting the presentation of atherosclerotic lesions evolution consecutive the apoptotic process [23], initial endothelial and smooth cells due to expression in excess of E_{as} antigene of endothelial cells. The evolution of the enzymatic proceses have been determinating the evolution of atheromplagues, those stables presented the weak lymphomonocytar neighboring reaction, towards differentiation by the richness of these inflamatories reactions of the neighboring athrom plagues in evolution. [10]

5. Conclusions

In myocardial affections induced by different causes, the apoptotic processes arised relatively frequent at 35% from myocitary cellularity (by some data), like in condition in which is studied especially in the entirely heart experimental material, in severs heart failure, in the extirpated hearts with a vieu to transplantation. The application of methods of molecular biology certified the existence of these lesions like apoptosis having the histological features and the ultrastructure above described also in intraoperatory myocardial biopsies.
The future researchers will be great amplified the numerous aspects not yet cleared up, uncerted, in the framework with the apoptosis and consecutive, the adequate therapy wich can be applied.

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