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CELLULAR BASIS OF AGING IN THE MAMMALIAN HEART

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Abstract

This review is concerned with the functional and structural changes occurring in the aging heart. These changes were investigated in an integrated fashion in Fischer 344 rats at 4, 12, 20, and 29 months after birth. Mean arterial pressure, left ventricular systolic and end-diastolic pressures, as well as stroke volume remained substantially constant up to 20 months. At 29 months, however, end-diastolic pressure was significantly increased, and dP/dt and stroke volume were depressed. Focal areas of interstitial and replacement fibrosis were markedly increased at 20 and 29 months, mostly in the subendocardial region of the ventricular wall. Also the aggregate number of mononucleated and binucleated cells in the left ventricle as a function of age was determined. The number of mononucleated cells increased up to 20 months but decreased thereafter; the binucleated cells showed a reversed pattern. The aging process of the heart involves a number of interrelated events including biochemical, electrical, mechanical and structural modifications. With aging and senescence, left ventricular failure develops in the Fischer rat model, and a similar process may occur in the human as well.

Key Words: Aging, heart, myocardium, left ventricle, fibrosis, hyperplasia, hypertrophy, nuclei, animal model.

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Introduction

One of the major difficulties encountered in the study of the effects of age on the cardiovascular system is the differentiation of the aging process itself from the presence of specific disease states. Atherosclerosis and ischemic heart disease are common events in humans and the severity of these pathological changes of the heart and blood vessels increases with age. Because the contribution of these variables to the alterations of the aged myocardium cannot easily be separated from the aging phenomenon alone, the changes in size of the heart throughout the life span of humans and animals species (Karsner *et al.*, 1925; Hegglin, 1934; Smith 1928; Reiner *et al.*, 1959; Reiner, 1968; Linzbach and Akuomoa-Boateng, 1973; Hutchins, 1980; Rakusan, 1984) are necessarily the result of multifactorial events in which aging plays an important but indistinguishable role. Furthermore, there is no temporal reference point yet that can be used to distinguish between maturational changes beyond sexual maturity and the aging changes *per se*, since they are both controlled by time as a critical factor (Weisfeldt, 1980a, b).

It is a general conviction that cardiac hypertrophy develops with age (Lakatta, 1979; Lakatta and Yin, 1982). However, it is still unknown whether the increases in mass of the left and right ventricles are brought about through hypertrophy of the myocyte population that is commensurate with or exceeds the growth of each ventricle. Proportional myocyte hypertrophy would imply that cell loss is not an important component of the detrimental role of aging on the myocardium, whereas a greater cellular hypertrophy would imply that myocyte loss is a significant aspect of the aging heart. Recent morphometric observations support the view that myocyte loss participates in the deterioration of the myocardium with age (Anversa *et al.*, 1986a, 1990a).

The work potential of the heart is controlled by the aggregate volume of myocytes and the magnitude of blood supply to the muscle cells. A proportional or disproportional adaptation of the capillary microcirculation with respect to the myocyte compartment of the ventricle

with age can be anticipated to preserve or alter the capillary parameters governing tissue oxygenation, namely, capillary luminal volume and surface densities, and the average distance from the capillary wall to the surrounding myocytes. These capillary parameters are functionally related respectively to the volume of capillary blood available for gas exchange within the tissue, the capillary area available for oxygen transport from the blood to the tissue, and the diffusion path length to the sites of oxygen utilization and ATP synthesis (Weibel, 1979; Hoppeler *et al.*, 1981). It can be assumed that the maintenance of these structural properties of the capillary bed in the myocardium will be associated with an adequate tissue oxygenation and functional capacity. On the other hand, decreases of capillary luminal volume percent and luminal area in the tissue, and an increase in the diffusion distance for oxygen (Olivetti *et al.*, 1990) may be accompanied by the development of myocytolytic necrosis with subsequent replacement and interstitial fibrosis and changes in the collagen content (Weisfeldt *et al.*, 1971; Medugorac, 1980; Lakatta and Yin, 1982; Medugorac and Jacob, 1983; Eghbali *et al.*, 1989; Olivetti *et al.*, 1990) and composition (Medugorac and Jacob, 1983) in the myocardium. These structural abnormalities may result in a reduced ventricular function potential (Capasso *et al.*, 1983; Anversa *et al.*, 1986a) that may progress into overt cardiac failure in the senescent animal.

Because of the importance of understanding the impact of structural modification of the aging myocardium on the functional capacity of the heart with senescence, quantitative morphology and quantitative physiology have recently been employed in an attempt to identify the critical events implicated in the deterioration of structure and function of the mammalian heart with aging.

Functional Characteristics of the Aging Myocardium

Growth of the left ventricular myocardium with maturation in the rat shows a well coordinated compensatory response up to 3-5 months after birth (Anversa *et al.*, 1986b). The capillary microvasculature, parenchymal cells and the subcellular components of myocytes all grow at a rate comparable to that of the increase in cardiac mass (Anversa *et al.*, 1986b, 1986c). Thus, physiologic hypertrophy takes place from childhood to adulthood and this growth adaptation appears to represent the compensatory mechanism that allows the heart to maintain normal pump function despite increasing workloads with postnatal development and maturation.

However, differences in heart size as a function of age complicate the interpretation of global functional measurements. Preservation of cardiac hemodynamics

Figure 1. Changes in mean arterial blood pressure, left ventricular end diastolic pressure, left ventricular peak systolic pressure and peak rate of left ventricular pressure development with age. * Indicates a value that is statistically significantly different ($p < 0.05$) from the values at the other age intervals.

Figure 2. Changes in stroke volume, heart rate, cardiac output and cardiac index as a function of age in 4, 12, 20, and 29 month old male Fischer 344 rats. *, **, + Indicate values that are significantly different ($p < 0.05$) from the corresponding result in 4, 12, and 20 month old animals, respectively.

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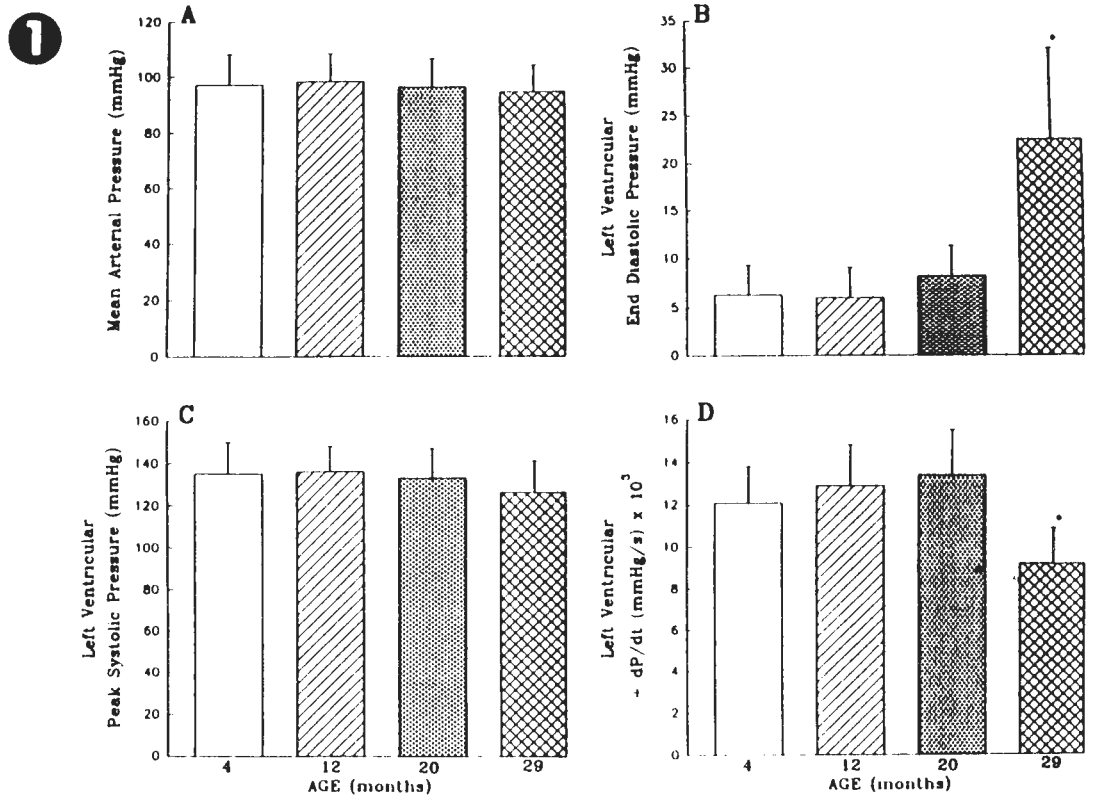
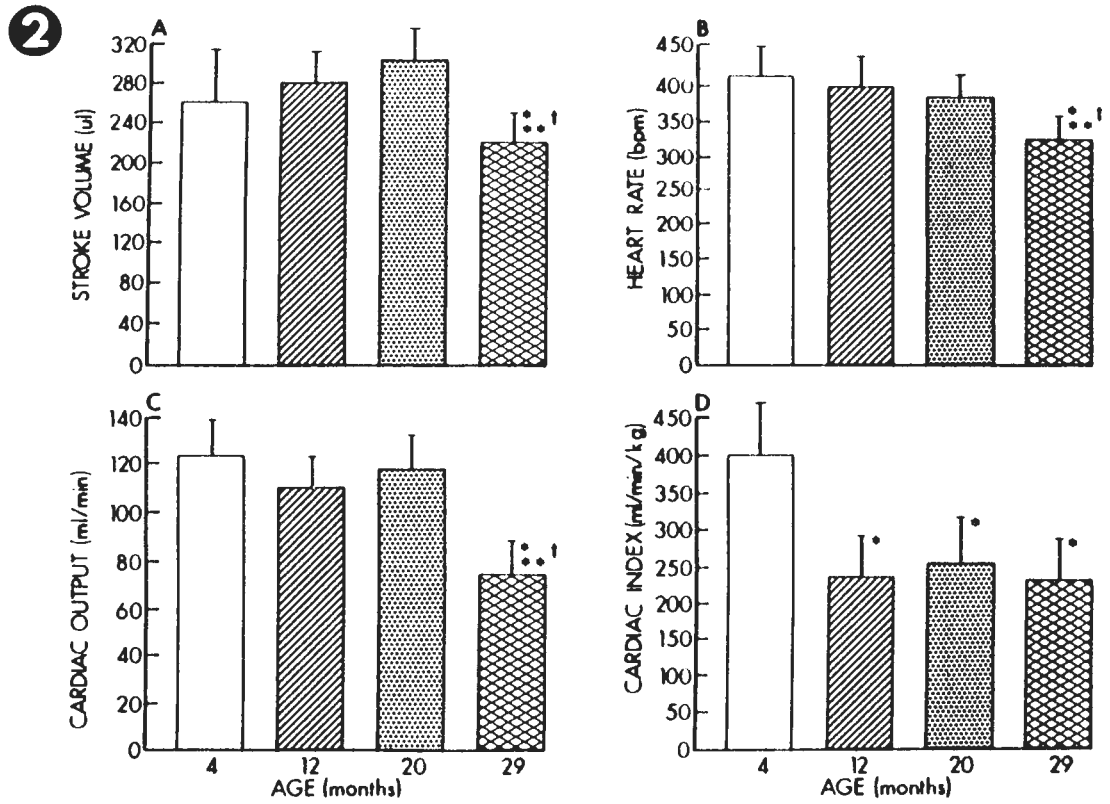
with age could be the result of greater mass with reduced contractile performance or greater mass and normal or enhanced contractile performance. Moreover, changes in tissue composition with potentially altered series elasticity may further complicate evaluating ventricular function in the intact heart. This formed the basis for *in vitro* studies of myocardial contractility, since the muscle may or may not have intrinsic mechanical abnormalities which are masked in the whole heart (Capasso *et al.*, 1982). However, the results accumulated in Sprague Dawley rats so far (Olivetti *et al.*, 1990) failed to reveal severe impairment of cardiac performance in both ventricles as function of age, with some exceptions. An age-related increase of time to peak tension and a decrease in the velocity of shortening and relengthening were found in the left myocardium. These *in vitro* parameters find their respective *in vivo* counterparts in the isovolumic and ejection phases of the cardiac cycle. Alterations in these mechanical properties can be expected to result in the generation of normal force levels (prolongation of time to peak tension), impaired ejection of systemic blood (depressed velocity of shortening), and abnormal ventricular filling (depressed velocity of relengthening). Changes in velocity of relengthening were also observed in right ventricular tissue. However, most of the isometric and isotonic timing parameters, force measurements, and indices of shortening were maintained up to, 19 months after birth.

In contrast with the observations above (Olivetti *et al.*, 1990), a progressive decline in the intrinsic mechanical properties of the myocardium was previously documented in the rat (Weisfeldt, 1980a, 1980b; Capasso *et al.*, 1983, 1986). A decrease in adenosine triphosphate activity (Alpert *et al.*, 1967; Heller and Whitehorn, 1972; Chesky *et al.*, 1983; Capasso *et al.*, 1983, 1986), an isoenzyme shift from V_1 to V_3 (Lompre *et al.*, 1981; Ebbrecht *et al.*, 1982), and a prolonged duration of the transmembrane action potential (Wei *et al.*, 1980; Lakatta and Yin, 1982; Capasso *et al.*, 1983, 1986) were also found to develop with age. These apparently


A
Mean Arterial Pressure (mmHg)
B
Left Ventricular End Diastolic Pressure (mmHg)
C
Left Ventricular Peak Systolic Pressure (mmHg)
D
Left Ventricular + dP/dt (mmHg/s) x 10³
4
12
20
29
AGE (months)


A
STROKE VOLUME (ul)
B
HEART RATE (bpm)
C
CARDIAC OUTPUT (ml/min)
D
CARDIAC INDEX (ml/min/kg)
4
12
20
29
AGE (months)

contrasting results may be explained by strain differences, since this physiologic and biochemical information was obtained in Wistar and Fischer 344 rats and was restricted to the left ventricle.

In summary, these studies indicate that the aging process of the heart in the rat is associated with the absence of severe mechanical abnormalities of the muscle mass, although the capacity of the myocardium to adapt to an added stress is markedly reduced (Guarnieri *et al.*, 1979; Capasso *et al.*, 1986; Isoyama *et al.*, 1987, 1988). Moreover, cell loss, excessive myocyte hypertrophy, inadequate capillary growth, scar formation, and indirect evidence of subendocardial ischemia have all been documented (Tomanek, 1970; Weisfeldt *et al.*, 1971; Anversa *et al.*, 1986d) to occur with the progression of life in the rat model. These interrelated events can be anticipated to produce profound changes in the anatomical determinants of the loading state of the myocardium: wall thickness and chamber diameter. Thus ventricular remodeling with wall thinning and chamber dilatation, in combination with unaltered diastolic and systolic ventricular pressures, may result in abnormal work loads on the myocardium at an age at which the growth reserve compensatory mechanisms of the myocyte and vascular compartments have been shown to be markedly depressed (Tomanek, 1970). These phenomena may initiate ventricular dysfunction and bear on its progression toward end-stage congestive heart failure. This possibility was tested in recent studies (Anversa *et al.*, 1990a; Capasso *et al.*, 1990) in which the functional and structural adaptations of the aging heart have been analyzed in an integrated fashion in Fischer rats at 4, 12, 20, and 29 month after birth. These age intervals were selected because they correspond to young adult, adult, aged, and senescent subjects in this animal model.

The changes in mean arterial pressure, left ventricular systolic and end-diastolic pressures and dP/dt in rats at 4, 12, 20, and 29 months after birth are illustrated in Figure 1. These physiological parameters were found to remain substantially constant up to 20 months. However, at 29 months, left ventricular end-diastolic pressure was increased by 3.57-, 3.75-, and 2.74-fold in comparison with values obtained in rats at 4, 12, and 20 months. These augmentations were all statistically significant (Capasso *et al.*, 1990). During the period from 4 to 29 months, left ventricular end-diastolic pressure increased from 6.3 ± 3.0 to 22.5 ± 9.5 mm Hg. Moreover, at the later age, left ventricular dP/dt was substantially depressed. When comparisons with the other rat groups were made, an average decrease in dP/dt of 31% was measured at 29 months. Thus, the marked elevation in left ventricular end-diastolic pressure, in combination with the depression in dP/dt, indicated that severe ventricular dysfunction had developed in rats at 29 months of age.

Figure 3. Changes in diastolic and systolic wall stress as a function of age in 4, 12, 20, and 29 month old male Fischer 344 rats. *, **, + Indicate values that are significantly different ($p < 0.05$) from the corresponding result in 4, 12, and 20 month old animals, respectively.

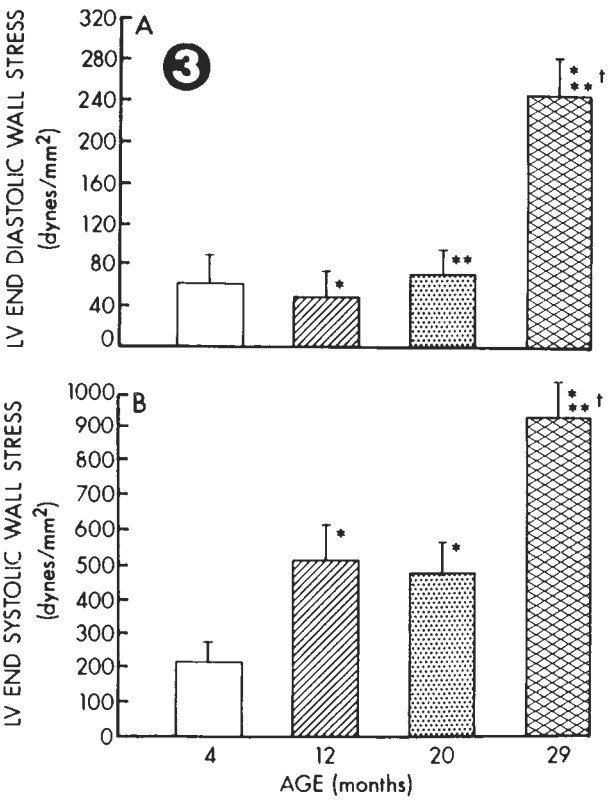
Figure 4. Changes in the volume percent of replacement fibrosis as a function of age in 4, 12, 20, and 29 month old male Fischer 344 rats. *, **, + Indicate values that are significantly different ($p < 0.05$) from the corresponding result in 4, 12, and 20 month old animals, respectively.

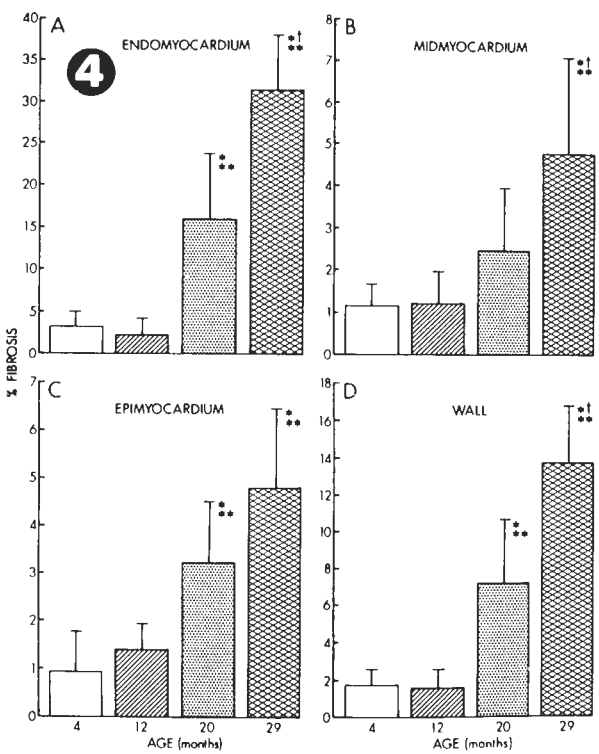
Figures 5 and 6. Number of mononucleated (**A**), binucleated (**B**) and total (**C**) cells in the left (**Fig 5**) and right ventricle (**Fig. 6**) as a function of age. * Indicates a value that is statistically significantly different from the corresponding result in 4 month-old rats. ** Indicates a value that is statistically significantly different from the corresponding result in 12 month-old rats. *** Indicates a value that is statistically significantly different from the corresponding result in 20 month-old rats.

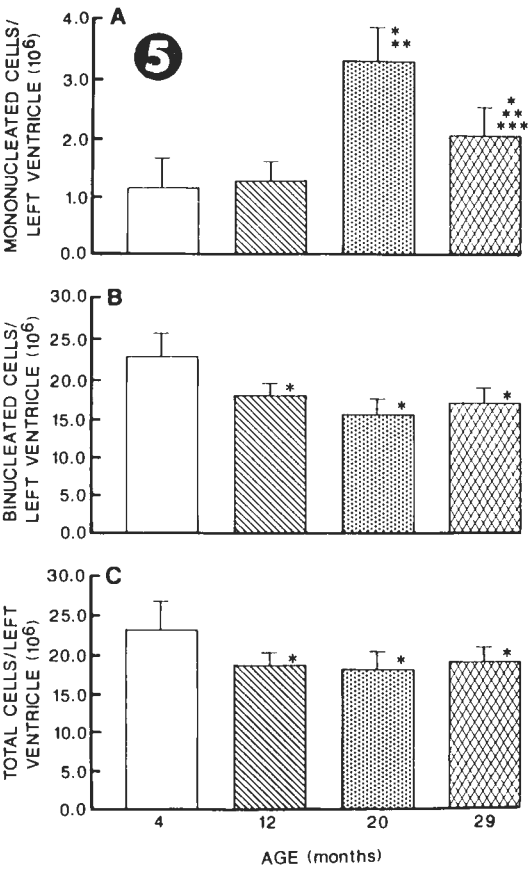
Additional parameters that further characterize the depression in the pumping ability of the heart with aging are illustrated in Figure 2. Stroke volume was identical in rats at 4, 12, and 20 months, but it decreased significantly by 27% in the senescent rats. Moreover, in the same group, heart rate was also diminished by 17% when compared with the younger animals, so that cardiac output was reduced by 39% from 4 to 29 months. Similar reductions in cardiac index, 40%, were measured in animals at 12, 20, and 29 months with respect to 4-month-old rats.

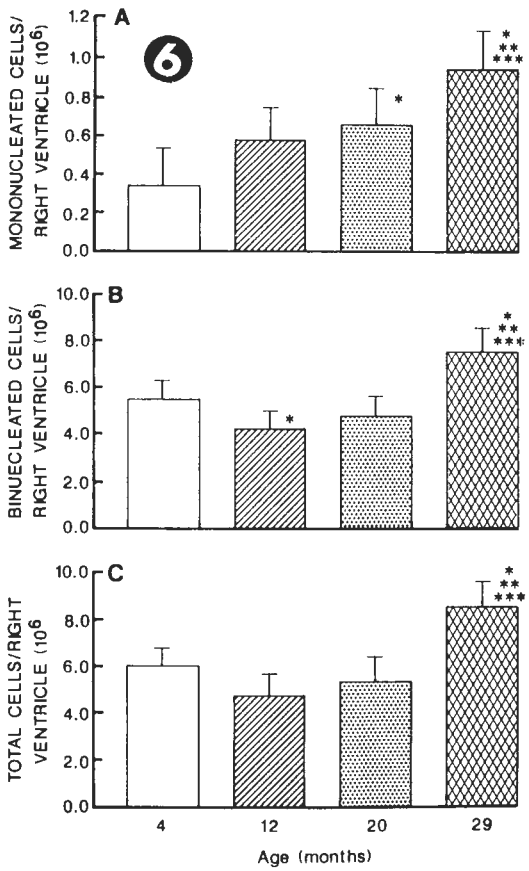
Figure 3 shows the changes in circumferential mid-wall end diastolic and peak systolic stress with aging. These measurements were computed from the parameters of wall thickness, chamber diameters and ventricular pressures (Capasso *et al.*, 1990). Diastolic wall stress was found to decrease initially from 4 to 12 months, from a value of 60 to 44 dynes/mm^2. However, increases in this parameter were seen in the subsequent two age intervals. Wall tension augmented by 65%, from 12 to 20 months, and by 239%, from 20 to 29 months. Moreover, mid-wall systolic stress was also observed to become higher as a function of age. Although values of systolic wall tension were comparable at 12 and 20 months, they were, on an average, 137% greater than the estimated value at 4 months. In addition, systolic wall tension at 29 months was 345%, 84%, and 92% larger than the corresponding determinations at 4, 12 and 20 months, respectively.

Importantly, when stress was calculated as a function of time during the cardiac cycle and location within


3
A
LV END DIASTOLIC WALL STRESS (dynes/mm²)
320
280
240
200
160
120
80
40
0
B
LV END SYSTOLIC WALL STRESS (dynes/mm²)
1000
900
800
700
600
500
400
300
200
100
0
4
12
20
29
AGE (months)


4
A
ENDOMYOCARDIUM
B
MIDMYOCARDIUM
C
EPIMYOCARDIUM
D
WALL
% FIBROSIS
4
12
20
29
AGE (months)


5
A
MONONUCLEATED CELLS/ LEFT VENTRICLE (10⁶)
4.0
3.0
2.0
1.0
0.0
B
BINUCLEATED CELLS/ LEFT VENTRICLE (10⁶)
30.0
25.0
20.0
15.0
10.0
5.0
0.0
C
TOTAL CELLS/LEFT VENTRICLE (10⁶)
4
12
20
29
AGE (months)


6
A
MONONUCLEATED CELLS/ RIGHT VENTRICLE (10⁶)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
B
BINUECLEATED CELLS/ RIGHT VENTRICLE (10⁶)
10.0
8.0
6.0
4.0
2.0
0.0
C
TOTAL CELLS/RIGHT VENTRICLE (10⁶
4
12
20
29
Age (months)

the ventricular wall, from 4 to 29 months, stress increased on the epicardial surface by 199% and on the endocardial surface by 77%. In addition, aging resulted in a progressive increase in the overall magnitude of stress on the myocardium. Increases in stress of 64%, 44% and 50% were found from 4 to 12, 12 to 20 and 20 to 29 months since the volumes dictated by the three dimensional surface configurations were 1.1, 1.8, 2.6, and 3.9 x 10^6 cubic units in the four age intervals examined (Capasso *et al.*, 1990).

In conclusion, left ventricular failure develops with aging and senescence in the Fischer 344 rat model and this observation indicates that a similar process may occur in the human heart as well.

Structural Characteristics of the Aging Myocardium

Light microscopic analysis of tissue sections of the ventricular myocardium of the Fischer 344 rat heart at 4, 12, 20 and 29 months (Anversa *et al.*, 1990a; Capasso *et al.*, 1990) revealed the presence of focal areas of interstitial and replacement fibrosis across the ventricular wall. Although scattered lesions of these types were noted at 4 months, they increased markedly with age, mostly at 20 and 29 months. Both ventricles were affected, and the impact of these changes resulted in a substantial accumulation of collagen within the myocardium. Moreover, these foci of replacement and interstitial fibrosis were more numerous in the subendocardial than in the subepicardial region of the ventricular wall.

Figure 4 illustrates the extent of fibrosis measured morphometrically in the endomyocardium, midmyocardium, epimyocardium, and as an average across the entire left ventricular wall, with aging. Although similar values were found at 4 and 12 months, the volume percent of fibrosis consistently increased during the remaining two time intervals in the different regions of the wall. Such a phenomenon of myocyte loss with healing and scar formation can be expected to be at least partially involved in both the thinning of the wall and the chamber dilatation encountered in aging rats. These tissue and cellular processes are important determinants of the loading state of the myocardium when interpreted at the cellular level of organization. Normal hemodynamics in the presence of a reduced number of ventricular myocytes creates an overload on the remaining viable cells that enlarge in the attempt to normalize cell stress (Anversa *et al.*, 1986d). It should be apparent that the pathological events of cell damage and repair and their impact on ventricular wall remodeling and myocardial performance are limited by the growth capacity of the viable cells. When the reserve of the cells to hypertrophy is exhausted, the condition of overload will compromise ventricular function leading to the development of congestive heart failure.

Although the factors responsible for myocyte loss with areas of fibrosis cannot be identified at present, local ischemia is the most likely possibility. Recent studies in the same animal model have shown that alterations in coronary blood flow hemodynamics develop with age (Hachamovitch *et al.*, 1989). Coronary vascular resistance has been found to increase, whereas coronary vascular reserve decreased, particularly in the subendocardial region of the left ventricular wall. This reduction in coronary blood flow potential could be the underlying etiological mechanism implicated in the occurrence of ischemic myocardial injury under stressful conditions. Moreover, the inadequate growth adaptation of the capillary microvasculature in aging-induced cardiac hypertrophy (Tomanek, 1970; Rakusan, 1984; Olivetti *et al.*, 1990), has been shown to produce a decrease in the endothelial surface accessible for oxygen exchange in the tissue and a greater average diffusion distance for oxygen transport to the myocytes (Olivetti *et al.*, 1990). These capillary characteristics may represent the structural template for local ischemia, resulting in scattered loss of myocytes in the ventricular wall of aging rats.

In conclusion, the aging process of the heart is characterized by alterations in the gross anatomical parameters of ventricular size and shape that, in combination with tissue injury and an elevation in ventricular wall stress, contribute to the development of cardiac failure in the senescent rat heart.

Aging and Myocyte Cell Number

A critical variable in the understanding of the aging process of the heart involves the quantitative estimation of myocyte number and its change with the progression of life in the senescent myocardium (Anversa *et al.*, 1986a, 1990a). By employing a recently developed methodology (Anversa *et al.*, 1990a) which requires the knowledge of the distribution of nuclei in myocytes and the determination of the total number of myocyte nuclei in the ventricular myocardium, the aggregate number of mononucleated and binucleated cells in the left ventricle as a function of age was determined (Figure 5). Mononucleated cells increased by 184% from 4 to 20 months. However, a 38% reduction in this quantity occurred from 20 to 29 months. The changes in the binucleated cell population followed a pattern opposite that observed in terms of mononucleated myocytes. Binucleated cells decreased by 21% from 4 to 12 months and 32% from 4 to 20 months but increased by 11% from 20 to 29 months. This latter change, however, was not statistically significant. In addition, at 20 and 29 months of

age, small quantities of trinucleated and tetranucleated cells were present. When the cell populations were added to yield the absolute number of myocytes in the left ventricle, it could be seen that myocyte loss occurred from 4 to 12 months, whereas in the subsequent age intervals, the increases in mononucleated cells first and binucleated cells later were capable of maintaining the total number of cells in the ventricle nearly constant. The aggregate numbers of trinucleated cells at 20 and 29 months were $0.11 \pm 0.71 \times 10^6$ and $0.59 \pm 0.051 \times 10^6$. Tetranucleated cells at 29 months were $0.16 \pm 0.024 \times 10^6$ cells.

The computation of the aggregate number of mononucleated cells in the right ventricle revealed that this cell population consistently increased with age, leading to an overall 2.65-fold (Anversa *et al.*, 1990a) increase from 4 to 29 months (Figure 6). Binucleated cells first decreased by 24% from 4 to 12 months but subsequently increased during the other two time periods. From 12 to 29 months, binucleated cells were augmented by 77%, so that the right ventricular myocardium at this age possessed 35% and 60% more binucleated myocytes than that at 4 and 20 months, respectively. Hyperplasia of mononucleated and binucleated cells as a function of age resulted in an overall 45% increase in the total number of cells in the right ventricle from 4 to 29 months of age (Figure 6). The number of trinucleated cells at 20 and 29 months was $0.020 \pm 0.014 \times 10^6$ and $0.228 \pm 0.0369 \times 10^6$. Tetranucleated cells at 29 months were $0.121 \pm 0.0, 191 \times 10^6$.

Although these data appear to indicate that myocyte cellular hyperplasia was restricted to the right myocardium, the magnitudes of cellular proliferation stated above can be considered to be only minimal indices of the actual extent of this cellular growth mechanism in the ventricles. The simultaneous presence of myocyte loss complicates the estimation of the real number of newly formed cells in the myocardium by any methodological procedure currently available. The process may initiate much earlier in life and affect both ventricles, but these events may be obfuscated by the concurrence of cell death (Anversa *et al.*, 1986a, 1986b, 1986c, 1990a; Tomanek and Barlow, 1990). The observation that the volume fraction of collagen in the myocardium continues to increase with age in the left and right ventricles and the fact that these changes are paralleled by an augmentation in the number of foci of replacement fibrosis across the wall strongly support the concept that myocyte cell death accompanies the progression of the aging process of the heart. Thus, myocyte loss results in an underestimation of myocyte cellular hyperplasia in the tissue, whereas myocyte hyperplasia leads to an underestimation of the magnitude of myocyte death in the myocardium.

It is well established that, once myocyte proliferation ceases by the age of weaning in the rat, both physiological and induced postnatal myocardial growth occur principally through hypertrophy of myocytes (Rakusan, 1984). DNA synthesis in cardiac muscle cells has been considered to come to an end between 2 and 6 weeks after birth in the mammalian heart (Claycomb, 1975, Dowell and McManus, 1978). In contrast, a hyperplastic component has been observed in cardiac enlargement produced in neonatal animals by nutritional anemia (Neffgen and Korecky, 1972) or after aortic banding in rats early after birth (Dowell and McManus, 1978). More recently, evidence has been accumulated that adult atrial (Oberpriller *et al.*, 1983) and ventricular (Olivetti *et al*, 1987, Anversa *et al.*, 1990a, 1990b) myocytes can be stimulated and that nuclear hyperplasia can be induced in cardiac muscle cells. Moreover, *in vitro* studies clearly demonstrate that DNA synthesis can be evoked in adult atrial and ventricular myocytes (Claycomb and Moses, 1985, 1988), pointing to the possibility that cardiomyocytes may retain their capacity to proliferate throughout life. Such a potential reserve mechanism has been suggested to be operative in the human heart (Linzbach, 1960; Linzbach and Akuomoa-Boateng, 1973). Moreover, growing information supports the view that nuclear hyperplasia reflects a comparable magnitude of cellular hyperplasia (Olivetti *et al.*, 1987, 1988). In addition, myocyte cellular hyperplasia can be elicited *in vitro* by different growth factors and tissue-type plasminogen activator (Claycomb and Moses, 1988), strengthening the concept that cellular proliferation may be induced in the adult heart *in vivo*. This cellular process may be present in the senescent myocardium in the attempt to maintain pump function in a failing heart, and it may constitute the ultimate response of the myocardium before intractable ventricular dysfunction and death supervene.

In conclusion, the aging process of the heart involves a number of interrelated events including biochemical, electrical, mechanical and structural modifications which all participate in the initiation of ventricular dysfunction and failure during senescence. Whether the observation that myocytes are not terminally differentiated cells and DNA synthesis in nuclei and cellular mitotic division can occur in the old heart offers new approaches for improvement of the deleterious effects of aging on the heart remains to be determined.

Acknowledgment

This work is dedicated to Dr. Renzo Laschi, who died in October, 1989.

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Discussion with Reviewers

S. Siew: You explain apparently contrasting results" documented by different investigations of the aging process in the rat heart "by strain differences". If results obtained in the Wistar rat cannot be extrapolated to the Fischer 344 strain, how can it be possible to extrapolate the findings in rat hearts to human hearts?
Authors: There is no perfect animal model which can really mimic the human disease. However, myocyte loss documented in Fisher 344 rats as well as in Sprague Dawley rats has recently been demonstrated in the human heart.

S. Siew: Another explanation given by you of the above apparently contrasting results" is that the work was "restricted to the left ventricle. Kindly, elucidate that statement. What are the differences between the left ventricles in the two strains of rats?
Authors: The mechanical behavior of the myocardium has been found to differ among rats of different strains as well as between the left and right ventricle. For example, the characteristics of the load dependence of relaxation are impaired as a function of age in the Fischer 344 rats whereas are essentially maintained in the Sprague Dawley rat. Moreover, with the progression of life, the left myocardium of the Fischer rat becomes less load dependent and the right myocardium becomes more load dependent.

S. Siew: How does "myocyte loss" result "in an underestimation of myocyte cellular hyperplasia"?
Authors: Myocyte loss and myocyte hyperplasia occurring simultaneously may compensate each other resulting in a constant cell number. Therefore, the absolute increase in cell number by proliferation may be fully masked by cell loss.

S. Siew: What proof do you have that the cells, which appear multinucleate at the light microscopy level, are not mononucleate cells with curvature of the nuclei, so that they are transsected more than once? Would DNA probes and confocal microscopy not be a more accurate means of investigating hyperplasia?
Authors: This possibility is not a realistic one. Myocyte nuclei are oval in shape. Moreover, cellular hyperplasia can only be documented by counting cells. Everything else is questionable.

S. Siew: Your findings appear to indicate that at the age of 29 months, all rats go into heart failure?
Authors: Yes, ventricular failure occurs at 29 months. However, increases in overall stress appear much earlier, at 12 months.

S. Siew: How do you relate your findings in the senescent rat heart to those of human "senile cardiomyopathy" (brown atrophy)?
Authors: The concept of brown atrophy at the organ level may not reflect the same phenomenon at the cellular level. The lack of accurate quantitative studies of the human myocardium with aging makes comparisons between animal models and the human heart difficult.

R.J. Tomanek: Proliferation of ventricular myocytes can occur by cytokinesis without karyokinesis. Do you consider this process to be likely in aging, i.e., binucleate cells undergo a lateral separation giving rise to 2 mononucleate cells (which can subsequently each become binucleate)?
Authors: This process is very unlikely. However, the possibility has to be raised an acknowledged. Our data clearly indicate that myocyte cellular hyperplasia occurs by mitotic division.

R.J. Tomanek: Why do you consider Fischer-344 rats to be good models for human aging. Why are they preferable to say Wistars or Sprague-Dawley?
Authors: Fischer 344 rats are maintained under controlled conditions throughout their life span and consequently, external influences are much more controlled. They are also raised according to NIH guidelines.

R.J. Tomanek: Would you speculate whether the aging alterations are intrinsic (i.e., simply time-related) or are they more likely related to stress over time?
Authors: This is a fundamental question that cannot be answered by the available information.