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OPINION

Rapid increase in human life expectancy: will it soon be limited by the aging of elastin?

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Abstract The postponement of the most frequent age-related diseases stimulated speculations of the possibility of "dying of old age". The selective decline of individual physiological functions—aging in spare-parts—indicates however the potential limitation of the life-span by the rapid decline of some of the vital parameters. We explored a possibility of such a limitation of maximal life-span by the age-related alteration of elastin, consisting in Ca-accumulation, lipid deposition and elastolytic degradation. The quantitative evaluation of these processes suggests an approximative upper limit for the elastic properties of the cardio-respiratory system of about 100–120 years, at least, as far as elastin is involved. This process, age-related alterations of elastic fibers, is however not the only one limiting the functional value of the cardiovascular system. Crosslinking of collagen fibers by advanced glycation end-products certainly contributes also to the age-dependent rigidification of the cardiovascular system. Therefore the answer to the initial question, can age-dependent alterations of a single matrix macromolecule be limiting such vital functions as the cardio-respiratory system—is a cautious yes, with however the caveat that other, independent mechanisms, such as the Maillard reaction, can also interfere with and limit further the functional value of such vital physiological functions.

Keywords Aging · Elastin · Vascular wall · Emphysema · Cardiovascular disease · Calcium · Lipids · Maillard reaction

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Abbreviations

ELR	The elastin laminin receptor
AGE-s	Advanced glycation end-products
TE	Tropoelastin
LOX	Lysiloxydase
mf	Microfibrils
MAGP	Microfibril associated glycoprotein
DSC	Differential scanning calorimetry
ROS	Reactive oxygen species
BLSA	Baltimore Longitudinal Study of Aging
LDL	Low density lipoproteins

Introduction

Average human life expectancy started to increase slowly more than a century ago and continued to increase much more rapidly over the last century. An

increase of about three decades is often cited (Robine et al. 1997; Riley 2001). Another important fact realized by epidemiologists is the recognition that the older age-cohorts experienced the most important increase in their life expectancy as exemplified also by the rapid increase of the number of centenarians, closely studied in France by Allard and Robine (2000) from the IPSEN Foundation and by Beregi (1990) in Hungary, to mention only those we could follow directly by participating in some of the biological studies. The recognition of these surprising facts started speculations proposing a continued increase in the average human life expectancy well beyond the known "records" as Jeanne Calment in France, who died in her 122nd year, which represent the actual maximal human longevity well correlating with the maximal life-support by elastin estimated around 120 years (see later). Several meetings were organised with such whimsical titles: "How to live beyond 150 years?" or even more, why not 180 or even 200 years! Let us cite as a "serious" example the publication by Oeppen and Vaupel (2002) arguing in favour of a continuous (and linear) increase of average life expectancy. These optimistic views were certainly encouraged by the reports of the team of Alvar Svanborg in Sweden (1988), who followed three cohorts of septuagenarians over more than 15 years and recorded what appeared to be a postponement of age-related diseases, accompanied by their decreased frequency and more favourable outcome as time vent on. A more cautious attitude was adopted by other epidemiologists as Olshansky and colleagues (1990) who pleaded for a levelling off of the increase of the average human life expectancy. They even predicted a decrease of life expectancy as a result of the epidemic dimensions of obesity in the US, followed closely by several European countries and spreading possibly even to developing countries. For these reasons it became interesting to deepen these reflections and ask the question, what does it mean to "die of old age", as predicted by some gerontologists rendered overoptimistic by the above mentioned results, justifying in medical terms the prediction of progressive, uninterrupted increase of human life expectancy. A word of caution came from pathologists, who carried out autopsies of centenarians and found regularly several pathologies, mostly cardiovascular diseases and malignant tumors, any of them could have killed the person well before 100 years (Johnson 1985). The above formulated question, how to live so long, could therefore be reformulated, asking how could the autopsied centenarians live so long with those fatal diseases? Let us cite one of the very first of these studies, published by Haranghy, founder of Hungarian gerontology (1965). His autopsies of centenarians revealed the presence of several severe pathological alterations any of them might have killed the person well before 100 years. Cardiovascular alterations were always present. This and similar results changed the meaning of the above cited question. It became more reasonable to ask what it really means to "die of old age"? Instead of claiming a continuous postponement of fatal diseases, it became more appropriate to ask the question, how did these centenarians (and many other old people) survive so long with the diseases discovered clinically and confirmed by autopsy. The next question we could ask is about the nature of deadly diseases which finally will end the life of most even optimally aging seniors? As far as malignancies are concerned, several studies indicated a decrease of frequency after 85 years (Macieira-Coelho 2001). We are left therefore with the cardiovascular, infectious and respiratory diseases, predicted to remain important for the 21st century. Infections might well continue to present a serious health hazard in an elderly population because of the weakened immune-defenses and frequent resistance of germs to antibiotics. Some time before the turn of the last century, the Harvard Public Health Institute published a forecast, cited by the Paris-edited International Herald Tribune, attributing a continued preponderance of cardiovascular diseases as the most frequent direct cause of death during the 21st century also. Recent rigorous statistical evaluations appear to confirm the "postponement" of age-related diseases and especially of cardiovascular diseases. An unforseen fact might however put in doubt the above cited forecasts, as we shall discuss it later. The progress in treatment of cardiovascular diseases was more rapid and efficient than that of malignant diseases. However up to the 1970s many practitioners avoided to treat hypertension of patients above 60 years of age, arguing that "they are old enough not to be bothered". The result was a high incidence of strokes and myocardial infarcts. A remarkable epidemiological study, also from northern Europe, showed convincingly, that the treatment of hypertension does significantly decrease the above

manifestations even in older individuals (Bulpitt and Fletcher 1992, for review). Postponement does not mean however the elimination of cardiovascular causes of death in "old age". A more relevant way to approach the above question is to analyse in detail the cellular and molecular factors involved in the age-dependent decay of the cardiovascular system. An overview of this approach will be attempted in this review.

Aging in spare-parts

It became evident over the last decades that the cellular-physiological functions of the organism do not all decline at the same rate with age (Weale 1993; Robert 1995). Beyond the individual variations, which also increase with age for most parameters examined [let us cite a well known example, the decline of cognitive functions highly variable from one individual to another, as shown, among other data, by the "Microcog" test (Powell 1994)], there is also a large variation in the speed of decline of individual physiological functions with age. Most of such individual differences (the SD of the mean value) increase with age. A number of such functions were shown to decline with highly variable speeds as a function of age, as shown by the collection of data by Weale (1993). A highly simplified graphical representation of these data consists of extrapolating the age-dependent decline of functions to the abscissa (Robert 1995) (Fig. 1). This shows the approximate age when the recorded individual functions reach the theoretical zero level. This approach represents a refinement of previous observations by Shock (1977) who also published rather detailed clinical observations on the differential decline of physiological functions. It appears from the collected data, that the faster decline concerns the elastic functions of the organism: the elasticity of the lens capsule, of the vascular system and also of the respiratory functions. It appears from the above cited data, that some vital functions decline fast enough with age to represent a serious obstacle, and finally a limitation to the survival of the organism, even if other physiological functions decline more slowly. This experimental fact, "aging in spare-parts" justifies the reformulation of our original questioning of the causes of "dying of old age" in the following terms: (1) can the decline of a single vital physiological function be fast enough to limit life expectancy, even if most other functions remain compatible with survival? and (2) can the age-dependent decline of a single cellular or molecular parameter be the rate-limiting step for the maintenance of a vital function? For a number of reasons we shall try to simplify these two questions by asking, if the aging of elastic fibers could be the rate-limiting step for the maintenance of adequate cardio-respiratory functions over life? The reasons of this reformulation of the above questions will be developed in the following sections of this review.

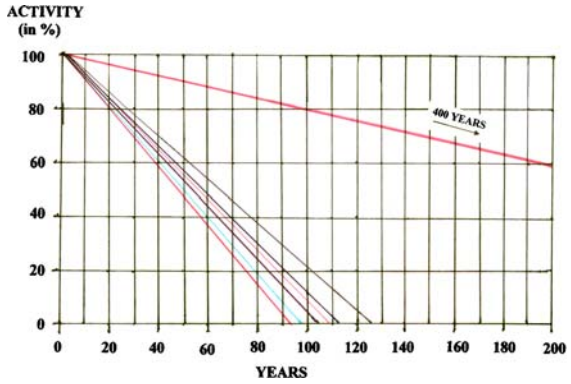

Fig. 1 Aging in spare-parts. The age-dependent decline of several physiological functions is plotted with extrapolation to the abscissa, indicating the approximate age when the functions reach the zero level. Data from Weale (1993) and Robert (1995). Functions, from the left (faster decline) to the right (slower decline): (1) elasticity of lens capsule; (2) glycosaminoglycans in cartilage; (3) maximal respiratory capacity; (4) elasticity of aorta; (5) rate of fibroblast proliferation; (6) memory; (7) speed of nervous conduction

Elastin and the vessel wall: from physiology to pathology

Physicochemical properties of elastin

The gene coding for tropoelastin (TE), the molecular precursor of elastic fibers appeared during evolution only with vertebrates (Sage 1982). Its crosslinking by the action of lysyloxidase (LOX) on a scaffolding of microfibrils was studied and described in great detail over the last half century (Mecham et al. 1995, for review). The microfibrillar scaffolding is composed of a number of individual glycoproteins, isolated first

from the elastic fibers by trypsin degradation and electronmicroscopic visualisation (Robert et al. 1971b). Fibrillins were shown to be the main components of microfibrils (mf) (Kielty et al. 2005), accompanied by several other glycoproteins as MAGP (microfibril associated glycoprotein), emilin and others. Their detailed description is beyond the scope of this review. The most relevant fact which concerns the present topic is the hydrophobic nature of elastic fibers, an important property which explains also their elasticity (Robert and Robert 1980, for review). Only a few basic and dicarboxylic amino-acids are present in elastin, more than 80% of its composing amino-acids are of a hydrophobic nature. Its rubber-like (entropic) elasticity is best explained by the strong decrease of entropy resulting from the increased exposure of hydrophobic amino-acid side chains to water in the stretched state as compared to the relaxed state. Although the decrease of librational degrees of freedom of the stretched peptide chains might also contribute (Urry 1980; see also Nemethy and Sheraga 1962a, b, c). An interesting observation by Partridge (1980) also contributed to this contention showing that during drying elastin looses its elasticity. In order to remain elastic at least 40% of water (on a weight basis) has to be present. The necessity of water to maintain the elasticity of a largely hydrophobic peptide chain pleads strongly for the predominant importance of the change of water structure in contact with the hydrophobic peptides to explain elastin's elasticity. The measurement of the specific surface of dried, micronised elastic fibers by radioactive Krypton-gas adsorption (Robert et al. 1970) pleaded also in favour of this contention, as well as the flow-calorimetric (Robert et al. 1971c) and differential scanning-calorimetric measurements (Bihari-Varga et al. 1983, 1986). The ultrastructural observations of Pasquali-Ronchetti et al. (1995), showing the close proximity of glycosaminglycans to elastin fibers in the aorta, might well be part of the explanation.

Aging of elastin

Age-dependent modifications of elastic fibers were studied in great detail over the last decades essentially because their involvement in the development of athero- arteriosclerotic modifications of elastic arteries (Robert and Robert 1980; Robert et al. 1986; Robert 1996, for reviews). The described modifications can be divided in three major categories: (a) fixation of calcium; (b) fixation of lipids; and (c) proteolytic degradation of elastic fibers. All three processes are progressive with age and are directly related to the peculiarities of the structure of elastic fibers.

Increase of calcium fixation

This process was studied by several laboratories, among them by Lansing (1959), preceded by Bürger (1947), both published age-dependent determinations of calcium in vessel-wall and in purified elastin from the large human elastic arteria. Figure 2 shows the data published by Lansing, showing that about 7% (on a weight basis) of calcium is saturating for elastic fibers. This figure shows also the rapid increase of the Ca-content with age of purified human aorta elastin. We should remind that the most frequently used purification procedure for elastin fibers involves heating in 0.1 N NaOH for 45 min to 100°C. Ca-fixation by elastin resists this treatment. According to Urry (1980), the β-turns of the peptide chains in elastin represent specific and high affinity fixation sites for Ca. Apparently the steady state level of calcium concentration in the interstitial fluid surrounding elastic fibers is sufficient to produce a progressive saturation of the available sites of elastin over the first six decades of human life, as shown on Fig. 2.

Figure 3 shows the result of Ca and cholesterol determinations in human aorta and femoral artery by the team of Burger (1947). The German authors confirmed the age-dependent increase of Ca in the elastic fiber of the large human arteries. Our studies on human aorta samples devoid of calcified plaques showed, that the major part of Ca in the vessel wall is strongly attached to elastin and remains bound to elastin fibers even after boiling in 0.1 N NaOH. A significant fraction of total recovered Ca extracted from human aortas appears however to be bound to the microfibrillar structures of the elastic fibers. It was shown since our above cited experiments, that repetitive sequences of fibrillin do bind Ca, in the so-called Ca-EGF-like repeats (Kielty et al. 2005). The contribution of fibrillin-bound Ca to total Ca fixed by elastic fibers is not known, and should also change with age according to the variation of the elastin to

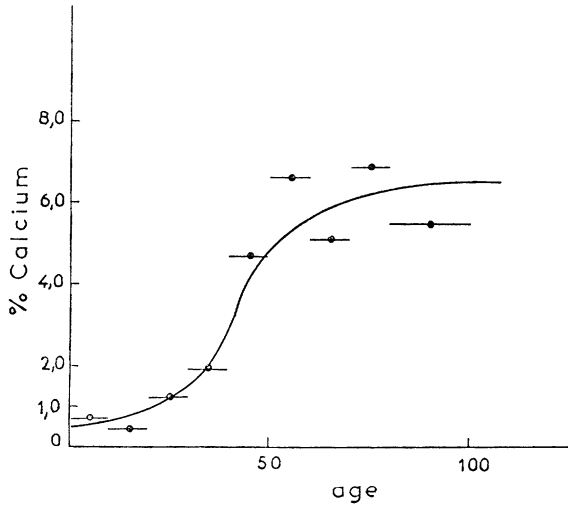

Fig. 2 Increase with age of the Ca-content of purified elastin fibers, drawn after table 5-2 (p. 146) from Lansing (1959)

microfibril ratio. It appears therefore, that the progressive increase of the Ca-content of elastic fibers with aging must be the direct consequence of the structure of elastin itself, and might well depend also on the age-dependent modifications of the conformation of elastin leading to increased exposition of available sites (see below).

Lipid fixation by elastin

The above cited data suggest a parallel increase of cholesterol and Ca in the wall of elastic vessels. This finding might well be the consequence of the specific physicochemical property of elastin, as demonstrated by Hornebeck and Partridge (1975) consisting in the potentiation of lipid uptake by elastin in presence of Ca. This interesting physicochemical property of elastin could be confirmed in our laboratory with Jacob et al. (1983). As shown in Fig. 4, when ^{14}C-cholesterol is shaken with micronised and lyophylised elastin fibers, the "uptake" of cholesterol (measured by washing the separated fibrils and determining strongly adhering radioactivity) was considerably increased in presence of Ca as compared to Na. Apparently Ca-fixation results in a transconformation of the elastin peptide chains, opening up more fixation sites for lipids and also for Ca. The affinity of elastin for lipids might be, at least partially the result of the hydrophobicity of the elastin peptide chains. Differential scanning calorimetry (DSC) experiments on fibrous and κ-elastin–fatty acid complexes showed a sharp transition enthalpy change with ΔH values of about 9–12 mJ/mg, independent of the chain length and saturation of the fatty acid chain (Bihari-Varga et al. 1983). The temperature where the enthalpy change occured, was lower with fibrous elastin than with κ-elastin and varied also with the chain length of the fatty acid. The same method, DSC, was used together with electron microscopy for the study of cholesterol esters bound to fibrous and κ-elastin (Bihari-Varga et al. 1986). The amount of cholesterol esters retained on elastin varied between large limits according to the nature of the fatty acid

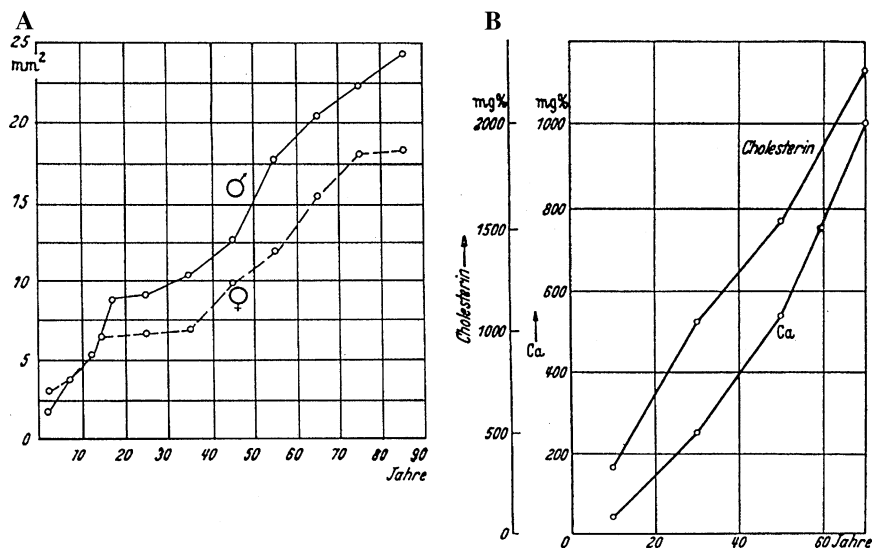

Fig. 3 (**a**) Increase of the wall thickness of human aorta for men and women (mm^2). (**b**) Calcium and cholesterol content of human aorta (mg%) Both as a function of age (years) on the abscissa. Reproduced from Burger (1947)

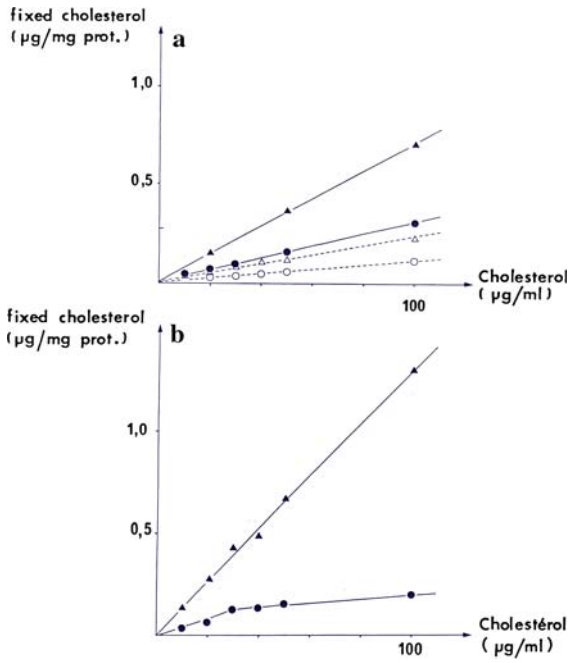

Fig. 4 Cholesterol bound by insoluble elastin (**a**) and by soluble elastin peptides (**b**) as a function of cholesterol concentration. (From Jacob et al. 1983). (**a**) insoluble elastin: ◯ in presence of sodium ions (0.18 M) at 37°C during 72 h; △ in presence of Ca ions (0.06 M) at 37°C during 72 h; ● in presence of sodium ions (0.18 M) at 65°C during 72 h; ▲ in presence of Ca ions (0.06 M) at 65°C during 72 h. (**b**) Soluble kappa-elastin: ● in presence of sodium ions (0.18 M) at 37°C during 72 h. The solutions are then allowed to coacervate at 65°C: ▲ in presence of Ca ions (0.06 M) at 37°C during 72 h. The solutions are then allowed to coacervate

esterifying cholesterol, between 0.5 µg/g elastin for cholesterol arachidonate to 240 µg/g elastin for cholesterol palmitate. The temperature of transition between the crystalline state and the liquid-crystalline state shifts to higher (above body-) temperature for cholesterol esters bound to elastin. At 37°C no liquid crystalline mesophase was observed with cholesterol oleate or linoleate–elastin complexes. For melting the crystalline structures temperature had to be raised to 51 and 42°C, respectively, for the cholesterol oleate and linoleate complexes. It appears therefore, that in the elastin-bound form the crystalline to liquid-crystalline transition is inhibited. This observation is in agreement with the presence of crystalline cholesterol deposits in advanced atherosclerotic lesions (Adams 1987). Electron microscopy showed a temperature-dependent thinning of elastin fibers in presence of cholesterol-oleate from 44.67 Å at 4°C to 34.90 Å at 37°C and to 34.36 Å at 58°C. For cholesterol-linoleate–elastin complexes the elastin fiber diameters shifted at the same temperatures from 48.80 Å at 4°C to 33.11 Å at 37°C and increased to 58.20 Å at 58°C. These results show that lipid fixation can affect the microstructural parameters of elastin fibers. Such changes might well modify also the susceptibility of elastin to elastases. The affinity of elastin to lipids is also strongly dependent on the conformation of the elastin peptide chains, modulated by their Ca-content. We shall cite only two experimental findings to demonstrate the importance of elastin–lipid interactions in the aging of vascular wall and atherogenesis, in in vivo conditions also. The first experiment pertains to the in vivo uptake of ingested ^{14}C-cholesterol by the rat aorta and its elastin fibers (Szigeti et al. 1972; Jacotot et al. 1973). A tracer-dose of radioactive cholesterol was injected in rats fed either a normal diet or a diet containing 4% cholesterol. As shown on Table 1, the ^{14}C-cholesterol content of the aorta was increased by 55% in the high cholesterol fed group as compared to the control group. The cholesterol uptake of the purified elastin (by NaOH treatment) increased by about 120%, twice as much as that of the whole arterial wall. The rapid and selective uptake of cholesterol by elastin was further increased by the high cholesterol diet. This parallels the in vivo increase of cholesterol during human aging until the 6th and 7th decades explaining the increased cholesterol content of elastin in the vessel wall, as described in the second experiment.

The second experiment to be cited in this context concerns the determination of individual lipid classes accumulated by human aortas and their elastin fibers (Claire et al. 1976) (Table 2). Determinations using gas-liquid chromatography were carried out on extracts of plaque-free portions of aorta-samples obtained at autopsies of individuals showing no or only very mild atherosclerotic alterations of their aorta (group 1), and of individuals with advanced atherosclerotic alterations (group 2). The successive aorta extracts as well as the purified elastin fibers of both groups contained relatively important amounts of all lipid classes (Table 2). Some of these lipids showed an important increase from group 1 to group 2 aortas (that is with progression of atherosclerosis), other lipids increased much less or not at all. The most interesting finding was however the strong increase (by 100%) of free fatty acids of a variety of

Table 1 Cholesterol content and radioactivity of rat skin, of subcutaneously implanted polyvinyl-sponge connective tissue, blood serum and aorta of animals on normal diet or on a cholesterol enriched diet (Szigeti et al. 1972)

Tissue	Normal diet			Cholesterol-rich diet		
	Radio-activity: cpm/g fresh tissue	Cholesterol mg/ g fresh tissue	Radio-activity: cpm/mg cholesterol	Radio-activity: cpm/g fresh tissue	Cholesterol mg/ g fresh tissue	Radio-activity: cpm/mg cholesterol
Blood serum	24,800*	1.24**	20,000	24,200*	1.37**	17,700
Sponge tissue	68,000	3.94	17,000	54,500	6.04	9,000
Skin	16,100	3.81	4,200	32,600	4.6	7,100
Aorta	12,000	1.07	11,200	18,600	2.42	7,700

Determinations on first and second pooled chloroform–methanol extracts. 0.5 mCi of ^{14}C-cholesterol (specific activity 143 × 10^3 cpm/mg) was administered with 10 kg of the diet given for 6 months, followed by the normal basal diet with ^{14}C-cholesterol for 30 more days. Total cholesterol content of the basal diet was 0.07%. Isotopic steady state was reached at the 18th day of the ^{14}C-cholesterol administration. For more details see the original publication

* cpm/ml of serum and ** mg/ml of serum: the * do not designate here statistical significance

Table 2 Lipid content of elastin isolated from human aortas with no or little atherosclerosis (Group 1) and from aortas with advanced atherosclerosis (Group 2)

Neutral lipids μg/g fresh tissue	Lipids extracted from elastin before enzymatic hydrolysis		Lipids associated with elastin, extracted after enzymatic hydrolysis	
	Group 1	Group 2	Group 1	Group 2
Free cholesterol	8.6	16	0.95	1.53
Esterified cholesterol	30.9	30.6	4.28	6.12
Triglycerides	78	154	7.1	6.9
Free fatty acids	48	204	10.2	20.1
Phospholipids	46	124	5.2	2.8
Total	211.5	528.6	27.73	37.4

Elastin was isolated by a fractional extraction procedure and lipids determined before and after the final purification step: boiling for 45 min in 0.1 N NaOH. For other details the original publication can be consulted (Claire et al. 1976)

chain lengths in elastin fibers from atherosclerotic individuals. Long chain fatty acids might well act as detergents modifying progressively the conformation of elastin peptide chains as their concentration increases within the elastin fibers. It can be concluded, that lipid and Ca uptake increase progressively during at least the first six decades of life, more rapidly in individuals with a life-style and nutrition favouring hyperlipidemia. The high affinity of elastin for Ca and lipids accounts for their accumulation in the vessel wall, essentially within the elastin fibers, as shown by the data on Tables 1, 2. This phenomenon contributes to and explains the well known atherosclerotic alterations found in elastic arteries with increasing age. As no recent, repeated results are available (no more autopsies performed), the effect of the recent increase of life expectancy on the above detailed parameters cannot yet be assessed.

Proteolytic degradation of elastin

This aspect of the age-dependent alteration of elastin fibers was intensively studied first by pathologists interested in the establishment and progression of the atherosclerotic process. Some pathologists as for instance Balo (1963) attributed a central role of elastin degradation in the vessel wall to the initiation and progression of the atherosclerotic process. The demonstration of a potential role for anti-elastin antibodies in the atherogenic process further strengthened this correlation (Robert et al. 1971a; Jacob et al. 1984).

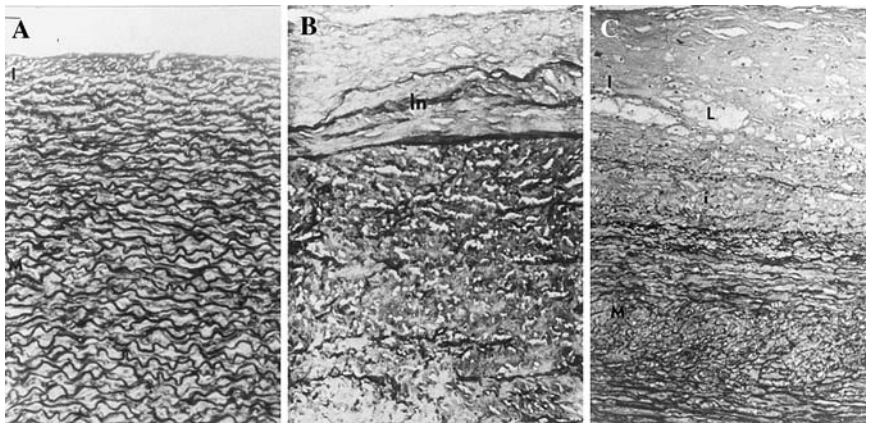

Fig. 5 Histological sections of human aortas showing the age-dependent lysis of elastic fibers progressing from the intimal side to the deeper parts of the media. (**a**) About 20 years; (**b**) about 60 years; (**c**) about 85 years (courtesy of Prof. H. Bouissou)

As shown on Fig. 5, age-dependent elastolysis is correlated with a progressive thickening of the intimal layer of elastic vessels like the human aorta, and a progressive fragmentation (loss of continuity) of elastin fibers (Robert et al. 1974). Several elastase-type endopeptidases were shown to participate in this process. MMP-2, MMP-9 and a membrane-localised serine-elastase on vascular smooth muscle cells appear to play a major role. As inflammation represents an important mechanism for the progression of atherosclerotic changes in the vessel wall, it seems quite probable that the elastase-type enzymes of mononuclear cells, shown to accumulate in atherosclerotic plaques and to express the elastin receptor (Péterszegi et al. 1997b) also contribute to this process. The activation of the elastin receptor by the liberated elastin peptides further upregulates this process (Archilla-Marcos and Robert 1933). The most relevant finding in respect of our present topic was the demonstration of the exponential increase

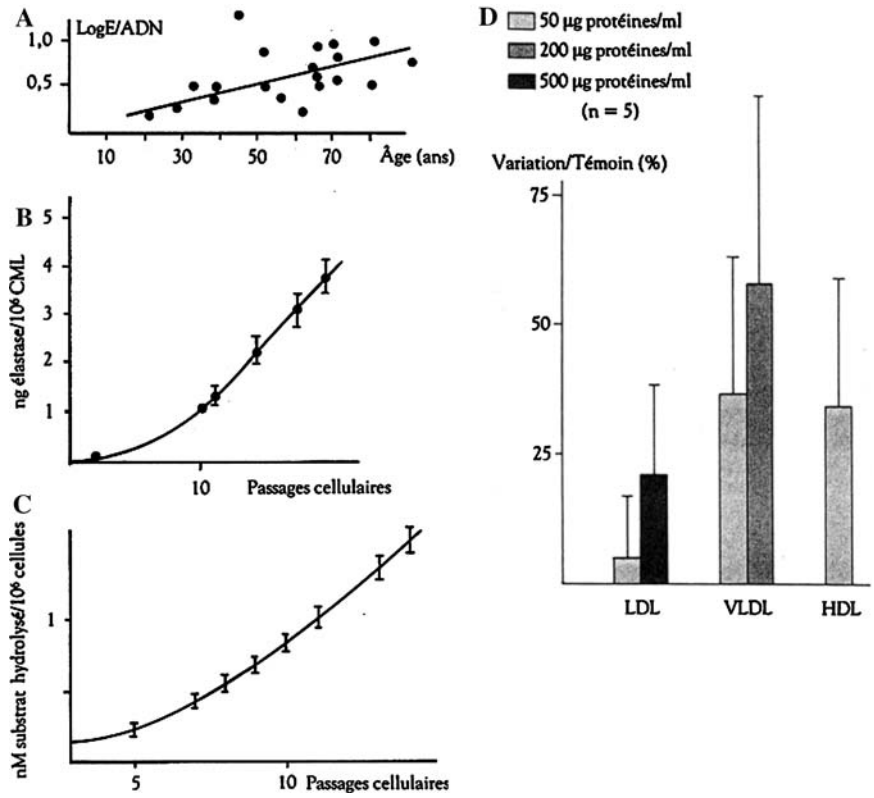

Fig. 6 Increase with age of elastase type endopeptidase activity in human aorta (**a**) expressed as log elastase per cell (DNA); (**b**) increase of elastase type endopeptidase activity with passage number for vascular smooth muscle cells; (**c**) same as B with skin fibroblasts; (**d**) effect of lipoproteins on elastase activity of vascular smooth muscle cells. Strong increase in presence of LDL and VLDL, no significant increase in presence of HDL. (Data from Robert et al. 1986)

with age of the total elastase-type endopeptidase activity of human aorta wall (Fig. 6) (Robert et al. 1986). Interestingly the progressive upregulation of elastase-type endopeptidases with increasing passages could be demonstrated in serial passages of vascular smooth muscle cells and skin fibroblasts also (Fig. 6). Here again it could be shown that hyperlipidemia increases the vascular elastase production. Aorta extracts from hypercholesterolemic rabbits (1% cholesterol in their food) exhibited a significantly increased elastase activity (Jacob et al. 1982). As shown on Fig. 6, LDL and VLDL (but not HDL) added to vascular smooth muscle cell cultures strongly increased their elastase-type endopeptidase production. Although "normal" lipidemia does not stop the increase with age of the elastase content of the human (or animal) vascular wall, hyperlipidemia strongly increased it by the above analysed three mechanisms. Ca-uptake, lipid uptake and elastolysis all contribute to the progressive loss of vascular elasticity and under the aging of elastic fibers.

Interaction between cells and macromolecules of the extracellular matrix is critically important in the pathogenesis of several diseases such as atherosclerosis. As mentioned before the degradation of elastin by elastases and even more when loaded with Ca and lipids during aging leads to the release of soluble peptides into the vessel wall and into the blood stream (Fülöp et al. 1990). Interaction between elastin peptides and cells was shown to be mediated by a cell-membrane elastin-laminin receptor (ELR) (Robert 1996). In this way the liberated elastin peptides contribute to the development and progression of atherosclerosis. Phagocytic cells, mainly monocytes play a key role in the pathogenesis of the atherosclerotic process (Péterszegi et al. 1997b). Extracellular matrix proteins and their proteolytic products have been shown to modulate cell mobility and metabolism (Ikeda 2003, for review). The demonstration that tropoelastin and the elastase digests of elastin are chemotactic towards fibroblasts, monocytes and neutrophils suggested that during pathological processes such as atherosclerosis characterised by increased elastolytic activity the elastin derived chemotactic peptides may be responsible for the monocyte/macrophage migration in the arterial intima (Senior et al. 1980). Although it is generally recognised that inflammatory processes are involved in the formation, progression and rupture of atheromatous lesions, the mechanisms that initiate these inflammatory responses are still poorly understood (Fülöp et al. 2001; Aikawa and Libby 2004). Elastin peptides could contribute by the above described mechanisms to the inflammatory process of atherogenesis: (1) by chemotactic attraction of monocyte/macrophages; (2) by induction of the production of free radicals and matrix metalloproteinases; (3) by induction of oxidation of LDL taken up in the vessel wall intima by phagocytic cells; and (4) by alteration of $NO^{\bullet}$ production. Altogether elastin and its degradation products might well contribute by their physico-chemical properties to the development and progression of atherosclerosis during aging.

Aging of elastin and the decline of the cardio-respiratory function: role of the elastin receptor

The above described mechanisms are mostly derived from in vitro and ex vivo experiments.

In order to infer to the in vivo situation, a number of complicating factors have to be taken into consideration. One of them is the interaction between cells and the extracellular matrix. Over the last decades of the 20th century it became well established that extracellular components, besides their mechanical role, take an active part in signaling to cells (Fülöp et al. 1992, for a review). This is the case for the cardio-vascular system also. The three major cell-types of the vascular wall, endothelial cells, smooth muscle cells and adventitial fibroblasts were all shown to express receptors mediating cell–matrix interactions. As the main emphasis of this review concerns elastin, mention should be made of the elastin receptor, "recognizing" apparently also laminin (ELR—elastin-laminin receptor) (Robert et al. 1989; Robert 1996, 1998; Fülöp et al. 1992, 2001). A number of physiopathological functions of this receptor were studied in our laboratory. Among them the most important functions concern the increased production of elastase-type endopeptidases and also reactive oxygen species (ROS), when the above mentioned vascular cells are exposed to elastin peptides (Fülöp et al. 2001). Such peptides are present in the circulating blood (Fülöp et al. 1990) and can therefore participate in the establishment of a vicious circle, increasing progressively the degradation of vascular elastin fibers (Fig. 7). This was shown to be

the case for T-lymphocytes also (Péterszegi et al. 1997a, b). Increasing concentrations of elastin peptides added to lymphocyte cultures produced an increased production of a serine-elastase followed by cell-death, first by necrosis and at a higher concentration of elastin peptides by apoptosis. Mononuclear cells in the human atherosclerotic plaques, obtained by endarterectomy, do express ELR and might well actively participate in the age-dependent degradation of elastin fibers and remodeling of the vascular wall as observed and mentioned previously (Péterszegi et al. 1997a, b). It is of note that during the aging process there is an uncoupling of the ELR receptors from their transmission pathway, as found in aging cells, which leads to uncontrolled elastin peptide and cell interactions manifested by age-dependent increase of free radicals and elastase production contributing to the progression of atherosclerosis (Robert et al. 1974; Robert 1996). The vicious circle mediated by the elastin receptor reacting to liberated elastin peptides is shown on Fig. 7.

In vivo consequences

Loss of vascular elasticity

In vivo measurements largely confirm conclusions drawn from the above detailed in vitro and ex vivo experiments. As shown by Bader (1967), O'Rourke et al. (1968) and Landowne (1958), the amplification of the pressure wave of human aorta, a measure of their elasticity, as well as the extensibility of the vessel, is linearly decreasing with age (Fig. 8). This can be largely attributed to the loss of elasticity of the vascular wall. This is however not the only age-dependent modification of the cardio-vascular system. The peripheral resistance increases progressively with age, as shown by Landowne (1958). These measurements concern to a large part the smaller blood vessels with only partial participation of elastin, much less predominant than in larger vessels, but present in smaller vessels also, except capillaries with no media anymore. Ultrasonographic exploration of the carotid arteries showed an age-dependent increase of wall thickness (Gariepy et al. 1995). This age-dependent increase, and as a consequence a rigidification, was significantly increased in hyperlipidemic subjects. Our (unpublished) results obtained with J. Chaudière and B. Jacotot, carried out with rabbits submitted to hyperlipidemic diets, more or less enriched in unsaturated fatty acids, showed a direct correlation between (morphometrically determined) degradation of the elastin fibers and the increased deposition of collagen. A similar mechanism might well explain the age-dependent increase of interstitial collagen in heart muscle with age (Folkow and Svanborg 1993, for review). Increased wall thickness must further exacerbate the consequences of the loss of elasticity of the vascular system. The progressive crosslinking of collagen and elastin by the Maillard reaction (advanced glycation end-products, AGE-s) further increases the rigidity of the vessel wall (Konova et al. 2004; Robert 2006).

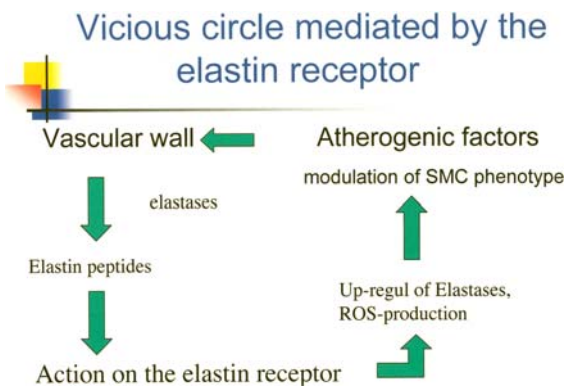

Fig. 7 Vicious circle mediated by the elastin-receptor. Elastin peptides liberated in the circulation act on the elastin receptor followed by upregulation of elastase and ROS production, further increasing elastin degradation

Decline of the respiratory function

Elastic fibers in the lung alveolar wall do also play a critical role in the efficiency of the cardio-respiratory system. Loss of alveolar wall surface with age as a result of ruptures of the thin alveolar wall, initiated by the lysis of its elastin fibers, under the development of emphysema, shown to increase with age. The role of elastolysis in this process was clearly demonstrated by the intratracheal instillation of elastase followed by the development of acute emphysema in golden hamsters (Cantor and Turino 1989). Here again long term clinical studies, especially those of the Baltimore Longitudinal Study of

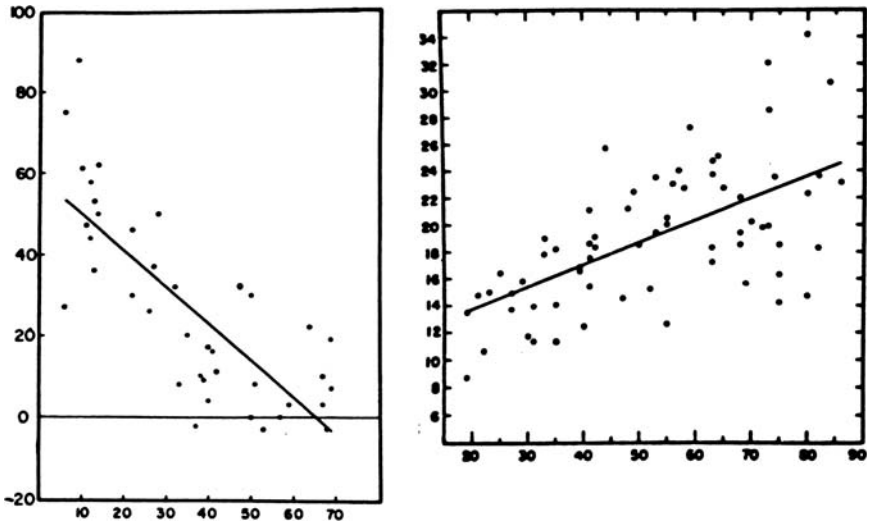

Fig. 8 Left: Amplification of the pressure wave of human aorta (%) as a function of age in years (O'Rourke et al. 1968). Right: Increased peripheral vascular resistance as a function of age in years (Landowne 1958)

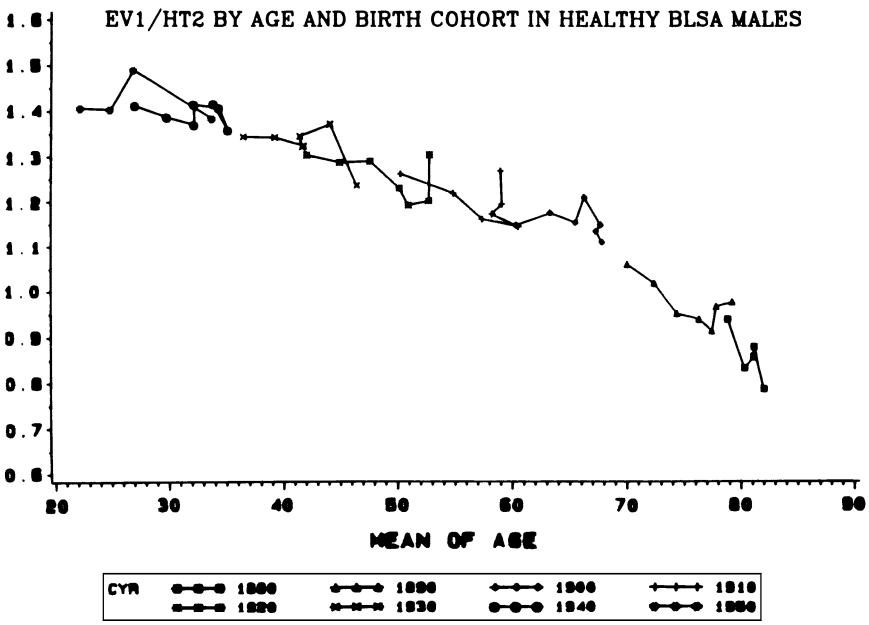

Fig. 9 Age-dependent decline of maximal respiratory capacity. Several measurements were done during the BLSA study on the same individuals, shown by the juxtaposition of broken lines. Ordinates: corrected vital capacity, EV_1/HT_2 (L). Abscissa: average age at examinations. The age-dependent decline was further confirmed recently (Fleg et al. 2005). BLSA: Baltimore Longitudinal Study

Aging (BLSA) clearly showed an age-dependent decline of the maximal respiratory capacity with age, even in non-smokers (Fleg et al. 2005). The data shown on Fig. 9 were obtained on a number of voluntary participants evaluated every 2nd year. The combined curves of the experiments can be extrapolated to the abscissa to suggest a total loss of respiratory capacity at around 100 years. These results were further extended by the Lakatta-team, showing that the age-dependent decline of the respiratory function is not linear but accelerates during later decades (Fleg et al. 2005).

Consequences for the heart

The 70 (or so) per minute contractions of the heart muscle must provide an efficient exchange of CO_2 for O_2 in the alveolar wall of lungs. The loss of contractile heart-myofibrils with age was demonstrated (Folkow and Svanborg 1993, for review) impairing progressively the efficiency of pumping by the aging heart. The loss of contractile efficiency is further aggravated by the progressive loss of vascular elasticity and increase of vascular resistance. A further aggravating factor is the increase of the collagen content of the heart

muscle, their crosslinking by the Maillard reaction decreasing the efficiency of the signal transmission for the rhythmic contractions, conjugated with age-dependent loss of cardiac responsiveness to β-adrenergic stimuli. The above described process should put an upper limit to the functional efficiency of the cardio-respiratory system close to the actually observed maximal human age-limit of 100–120 years. This age limit has to be considered as an approximation because of the age-dependent increase of the dispersion of individual values around the average for most physiological functions. Only a significant inhibition of the above-described processes could further limit the age-dependent loss of the elasticity of the cardio-respiratory system. The last decades of human life are certainly accompanied by a rapid loss of adaptability of the cardio-respiratory system even to moderate efforts, which is one of the major characteristics of the aging heart. Both the catecholamine or exercise induced increase in heart rate and myocardial contractility are definitively blunted in elderly subjects. Thus for cardiac output to be increased in proportion to the body's metabolic needs despite inadequate contractile and chronotropic reserves, the aging left ventricle mainly engages the Frank Starling mechanism. This leads to the diastolic dysfunction observed with aging especially during effort. We also mentioned the importance of life-long nutritional factors which might well further limit (or slightly increase) cardio-respiratory efficiency. Continued physical training, regular exercise were also shown to delay the decline of the aging cardio-respiratory system (Grounds 1998). Regular exercise—running in a turning wheel for rats—was shown to delay the age-dependent modifications of large vessels. All these considerations render difficult a more precise calculation of an upper age-limit of cardio-respiratory efficiency. A further complicating factor is represented by frequent broncho-pulmonary infections, leading to increased intraalveolar elastase release by mononuclear cells, accelerating loss of respiratory function. In well-trained individuals an efficient left-ventricular function can apparently delay the fatal loss of cardio-respiratory function.

Possibility for pharmacological interventions

Beyond the long-prescribed alimentary precautions and physical exercise more efficient pharmacological interventions might well provide a further delay for the decline of the cardio-respiratory function in aging individuals. The efficiency of statins for the control of lipidemia is certainly of importance. The protection of elastin fibers from proteolytic degradation, also against lipid- and Ca-deposition is well within the realms of an efficient pharmacological approach. Treatment with the antiplatelet drug aspirin can also be mentioned in this context, not only for its antithrombotic but also for its anti-inflammatory action. The rapidly increasing elderly population in industrialized countries could render an approach to elastin-protecting treatments economically rewarding for the pharmaceutical industry.

The Maillard reaction

In this review we tried to answer the question, raised in the Introduction, if the age-dependent modifications of one macromolecular component, namely elastin, could put an upper limit to the function of vital physiological processes such as the one fulfilled by the cardiovascular and respiratory systems; The answer is a cautious "yes" because of the exceptionally important role of elasticity both for the vessel wall and for the lung alveoli. The age-dependent alterations of the elastic tissue has also as a consequence the aggravation of related cellular and molecular functions as exemplified by the above discussed role of the elastin-laminin receptor. This does however not exclude the potential role of other mechanisms. This is the case of the Maillard reaction as shown by the multiple harmful effects of the advanced glycation end-products (AGE-s) (Ikan 1996; Baynes et al. 2005; Robert 2006, for reviews; Konova et al. 2004). These substances act also by two different but interrelated mechanisms. One is the increasing rigidity of the macromolecular structure of the cardiovascular and pulmonary systems, the result of the reaction of AGE-s with amino-groups (essentially ε-amines of lysine) on collagens, elastin and other macromolecules (Ikan 1996; Baynes et al. 2005 for reviews). The other is the chain reactions mediated by the interaction of AGE-s with their receptors, triggering further ROS-mediated harmful effects including DNA-damage, attributed also to glycoxydation. These reactions will certainly aggravate and limit further the functional value of the

cardio-respiratory system, without however negating the above developed arguments tending to substantiate a positive answer to our initial question.

Conclusions and epistemological reflections

The above summarised experimental data do not seem to substantiate an important further extension of average human life expectancy (see also Olshansky et al. 1990). If most "seniors" would accept the discipline of healthy food-intake (as for instance the Mediterranean diet) and physical exercise, it might be possible to shift average life expectancy toward the recorded maximal values of an increasing fraction of the senior population. The actual trend of overeating and replacing physical activity by watching television does not favour such an optimistic forecast. Remains the hope of innovating pharmacological interventions justified by competent economists to stimulate further investments by major pharmaceutical firms in the above described field, well beyond the control of hypelipidemia. However, healthy life-style would not be able to nullify all the changes occurring with age due to epigenetic and posttranslational processes (Robert and Miquel 2004).

As far as the above detailed mechanisms of elastin aging are concerned, it should be emphasised that all of them belong to the post-genetic, post-translational phase of cellular biological processes. The gene coding tropoelastin, result of an important evolutionary step at the level of vertebrates (Sage 1982) largely determines not only the elastic properties of the crosslinked subunits, the elastin fibers, but also their high affinity for lipids and calcium. Their progressive accumulation within the elastin fibers results in the age-dependent decrease of their elasticity and facilitates their degradation by elastase-type proteolytic enzymes. The liberated elastin degradation products, acting on the elastin-receptor, will trigger a further amplification of this process. It is hard to resist the temptation claiming the "neglection" by evolutionary processes of the protection of vital functions from their age-dependent deterioration. Intake of Ca and lipids as well as sugars is necessary for survival. Besides their vital functions they participate in the age- and time-dependent decline of vital functions as those of the cardio-vascular and respiratory systems. Evolution of vertebrates (and possibly of other organisms) largely neglected the development of devices rendering vital functions resistant to the direct detrimental consequences of their structure as determined by the genes coding their primary aminoacid sequence. Mechanisms involved in the aging of elastin as well as the Maillard reaction represent good examples of this principle. Even if the aging of elastin would not represent the only rate-limiting factor for the age-dependent decline of the cardio-respiratory system, it certainly plays a sufficiently important role to put a limit to functional performance of these vital systems and designate them as primary candidates responsible for "dying of old age" (Robert and Miquel 2004).

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