The process of ageing reflected by histological changes in the skin

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Abstract
Structural and functional alterations, as well as changes occurring in the aspect of the skin during the ageing process, are due to some complex mechanisms, determined by intrinsic and extrinsic factors, which act synergistically. This study aims to analyze the histological changes of collagen and elastic fibers and of skin vasculature that occur in time, during the process of ageing. Fragments of skin have been collected from subjects of both sexes and of different ages, totaling 121 samples from different body regions. The following stains were used: Modified Goldner’s Masson trichrome, Ferric Orcein–Hematoxylin and the combined stains Orcein–Goldner’s trichrome. As the process of ageing advances, collagen fibers become thinner and change their aspect; at advanced age, the lysis of collagen fibers and their thickening in the deep dermis is present, as they become more fibrous. Elastic fibers show the tendency of fragmentation at more advanced age, gradually change their tinctorial affinity and reduce in amount; in the deep dermis, they tend to thicken progressively, in the presence of discrete elastolysis processes that evolve steadily and irreversibly. Thus, processes of elastic fibers degeneration and lysis run faster by comparison with those of collagen fibers degeneration. With ageing, a progressive reduction of dermis vasculature is present, due to a reduction in the number and size of vascular vessels, which is in its turn associated with the progressive alterations of vascular walls components, changes that advance until the function of the vessel ceases.

Keywords: skin, dermis, histological changes, elastic fibers, collagen fibers, vasculature.

Introduction
The theories emitted about the mechanics of the aging process are numerous, with different approaches and solutions [1, 2]. Structural and functional alterations coupled with modifications in the aspect of the skin in the aging process are due to complex mechanisms started by intrinsic and extrinsic factors, which act in synergy [3]. There are a number of studies that followed the causes which determine and accelerate the aging process of the tegument, its effects on its functionality and the intensity of structural modifications at the morphological level [1, 4]. At tegumentary level, once the aging process commences there are degenerative processes of collagen fibers and elastic fibers taking place [1, 5]. The histological analysis of vascularization points out a tight link between the tegument, deserved tissue and senescence [6].

The study follows the effects that the aging process has on the epidermis and dermis and the level of alteration of collagen fibers and elastic fibers on the calibration of blood vessels and tegumentary vascularization.

Materials and Methods
Histological examination is one of the most important methods of accurately highlighting the structural changes that occur in the skin.

The prospective study was undertaken at Emergency County Hospital of Oradea, Romania, between February 2009–August 2011. In order to ensure a range of subjects that would be as diverse as possible, fragments of skin from patients of both sexes and of different ages were taken during surgical interventions. Subjects were aged between 2 and 85 years, a total of 121 samples being taken from different parts of the body: the head, especially the face, the back, the upper and the lower limbs.

The tegumentary biopsy of at least 4 mm was fixed in a solution of formaldehyde 10%, with a 10 times bigger volume than the extracted piece. The time of fixation was on average 1–2 hours for each mm of thickness of the cutaneous piece.

Several special staining techniques were used in order to highlight a large number of elements: collagen fibers, elastic fibers, blood vessels. We have used the Orcein–Goldner’s trichrome. Orcein acid especially points out elastic fibers, which seem brown-red [7]. The Goldner’s trichrome coloration is effective on collagen fibers and is known as Masson’s trichrome, changing the color of nuclei to coffee-black, of muscle fibers, creatinine and cytoplasm to bright red, collagen and mucus to bluish green erythrocytes to yellow/orange [7].

Coloring kits from the DiaPath S.p.A. (Italy) were used. Sections were undertaken at 3–4 μm using Microm Series HM325. Histopathological examination was made using a Nikon Eclipse E200 microscope. Pictures were taken using a Nikon Coolpix 4500 camera.

Results
Structural, histological changes of the skin that occur during the ageing process are complex and influence all skin components. Structural differences, that influence all skin layers and its annexes, differentiate the skin of
elder persons from that of young individuals. In the realization of the study, biopsies from subjects of different ages were collected (Table 1) observed the evolution of structural changes in the skin and its conjunctive components.

Table 1 – Distribution by age groups of the number of taken biopsies

<table>
<thead>
<tr>
<th>No.</th>
<th>Age group [years]</th>
<th>No. of taken biopsies</th>
<th>Distribution in percent</th>
</tr>
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<tbody>
<tr>
<td>1.</td>
<td>2–20</td>
<td>7</td>
<td>7.43%</td>
</tr>
<tr>
<td>2.</td>
<td>21–40</td>
<td>13</td>
<td>10.74%</td>
</tr>
<tr>
<td>3.</td>
<td>41–50</td>
<td>18</td>
<td>14.87%</td>
</tr>
<tr>
<td>4.</td>
<td>51–60</td>
<td>24</td>
<td>19.83%</td>
</tr>
<tr>
<td>5.</td>
<td>61–70</td>
<td>23</td>
<td>19%</td>
</tr>
<tr>
<td>6.</td>
<td>71–80</td>
<td>22</td>
<td>18.18%</td>
</tr>
<tr>
<td>7.</td>
<td>≥81</td>
<td>12</td>
<td>9.91%</td>
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</tbody>
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Skin areas where from anatomical samples were taken: face, the anterior thorax, the posterior thorax, arms, palmar part of the hand, finger pulp; as regards the lower limbs, biopsies were taken from the thigh and the calf region.

The conjunctive components of the dermis suffer a series of changes. Thus, our study has revealed that the superficial dermis of people under the age of 50 years presents a relatively loose structure. Collagen fibers are prevailing quantitatively and, when observed at microscope, they appear relatively thin and with different orientation, so that they intersect at different levels. Deep dermis looks denser than superficial dermis, where collagen fibers appear definitely thicker and, concerning their size, they have the aspect of typically dense, non-oriented conjunctive tissue. After the age of 50 years, especially in the first five years, the large majority of the aspects presented above continue to be present in a similar form, although there are changes, discrete at first, but which become more obvious with ageing (Figure 1).

Collagen fibers suffer changes. The first changes, detectable by optical microscopy investigations, also appear after the age of 50 years, which demonstrates that the ageing process of both types of fibers begins at about the same age, and runs somewhat parallel to the changes affecting elastic fibers but the speed of the process and its consequences on the structure and function of the skin are largely different. In the first stage, the thinnest fibers are affected, then the process is extended, so that more and more superficial dermis fibers undergo fragmentation processes and finally lysis. Therefore, the gradual lysis of connective fibers determines a gradual thinning of the superficial dermis; even more its aspect changes so that it becomes more dense and the collagen fibers it contains appear thicker than normally encountered at this level. The thinning is clearly due to the quantitative reduction of collagen fibers, while the increased density can be associated with the fact that first the thinnest fibers are lysated, so that they disappear almost completely, and for a while only those that are slightly thicker remain. From this point on changes can be observed at the level of the deep dermis as well. More exactly, collagen fibers start to thicken – up to a certain point, this renders a certain density to the deep dermis. It seems that, in this way, the resistance of the skin, which becomes weaker at surface, is ensured, so the thickening of collagen fibers in the deep layer appears to be a compensatory response. Subsequently, fragmentation processes of collagen fibers in the deep dermis can be observed, alongside a tendency of slow, though progressive amplification of collagen fibers fragmentation and lysis that occurs with age. The results obtained through the microscopic study indicate that the lysis of a number of collagen fibers determines the appearance of spaces between the remaining fibers, which are small at first but later grow in size, so that overall the deep dermis appears to be composed of thick collagen fibers, but these are not dense as regards their distribution. Collagen fibers appear thicker and thicker at people of about 70 years, so the deep dermis becomes more and more fibrous in appearance.

The histological study shows that, in some people over the age of 75–80 years, several particular aspects of collagen fibers appear in the deep dermis. These particular aspects indicate the tendency of separation of the fibrils that make collagen fibers, which in this case appear thick and even very thick, but with a rarefied, broken aspect, which has repercussions upon the function of these fibers – they are by no means as strong as the ones with a dense structure. Collagen fibers appear very thick, but gradually lose their typically dense aspect, becoming more and more loose and teased in appearance, in other words the fibrils that make them become more and more obvious (Figure 2).

It seems that the process will not stop here, but will continue affecting more and more components of the dermis from the structural point of view, which will have direct consequences on the function of resistance that dermis ensures to the skin. It should be pointed out here
that these latter aspects were observed only in some of the cases studied.

Important and obvious changes also occur in elastic fibers, so that in subjects aged less than 50 years the elastic fibers appear thin and relatively long at the level of the superficial dermis, being oriented in several directions, mostly parallel to the skin surface (Figure 3).

Figure 2 – The trend towards disorganization of collagen fibers, with an emphasis of fibrils (Orcein–Goldner’s trichrome stain, ob. ×40).

In the deep dermis, elastic fibers appear somewhat thicker and longer, most of them being arranged parallel to the surface of the skin. After the age of 50 years, several changes may be observed which, though subtle at first, become more obvious with age. In the beginning there is a tendency to fragmentation of a relatively small number of elastic fibers, both at the level of the superficial and of the deep dermis (Figure 4).

Figure 3 – Thin elastic fibers in the superficial dermis, most of them being oriented parallel to the surface of the skin (Orcein–Goldner’s trichrome stain, ob. ×25).

After the age of 55 years, it is found that the superficial dermis gradually loses its lax appearance and is thinning gradually. Elastic fibers appear more and more fragmented (Figure 5) because at an older age, they gradually change their tinctorial affinity (Figure 6) and even visibly reduce quantitatively. Changes also occur in the deep dermis, i.e. elastic fibers tend to thicken gradually (Figure 7); simultaneously elastolysis processes are recorded, which are discrete at the beginning but develop progressively and irreversibly with age.

The aspect of fragmentation becomes more and more obvious during the process of ageing (Figure 8) and it was found that few fibers maintain a relatively elongated appearance. At the same time, there is a more pronounced thickening of elastic fibers, both the fragmented ones and those that retain a certain fibrillar aspect.

Gradually, the fragmentation process practically influences all elastic fibers. At people over 70 years, all elastic fibers appear fragmented, many fragments presenting tinctorial affinity – which is more or less modified (Figure 9) in that, observed at a microscope, fibers appear more weakly stained with Orcein, which shows that, besides fragmentation, they suffer structural modifications as well, presenting a gradual loss of elastic fibers typical structure. Progressive processes of elastolysis determine the gradual disappearance of elastic fibers as an entity.

Histological investigation revealed quite important differences in some cases as regards skin vasculature, and all aspects suggest a direct relationship with the processes of skin ageing. Thus, research results show that in subjects less than 50 years, with normal aspect of the dermis, the vasculature is directly related to the tissue it serves. The superficial dermis appears highly vascularized and numerous small caliber vessels are present here.

In areas with thicker epidermis, where the superficial dermis presents papillae, vasculature is very well represented (Figure 10). The higher the number of cell layers at the level of the epidermis, the more demanding their nutrition is, which requires a larger exchange surface (Figure 11). When the superficial dermis begins to show subtle changes at the level of its fibers, the first changes in blood vessels may be observed. At this stage, i.e. at the onset of the ageing processes, it is not necessary to observe significant changes in the number of vessels and their structure, but there are discrete, yet progressive changes with regards to the vessel size, namely that the diameter
of the vessel is reduced gradually. The study results show no significant reduction in the number of vessels, even in more advanced stages of ageing, as long as the superficial dermis remains relatively lax in appearance. Even when the superficial dermis gradually loses its lax appearance, but still retains its papillary aspect, it remains relatively well vascularized (Figure 12).

When the superficial dermis gradually loses its papillary aspect, it becomes thinner and reaches an increasingly dense aspect and blood vessels gradually reduce not only in size but also numerically, and as the process of senescence progresses, they become small and rare that is numerically reduced (Figure 13). It follows that the progressive reduction of dermal vasculature is expressed, from the morphological point of view, through size reduction and progressive numerical reduction. The latter is due to the occurrence of changes in the vascular wall components, changes that advance gradually with age, until the vessel ceases to function. In the first phase, there are changes in the endothelial cells, which initially translate by granular degeneration, discrete at first but subsequently more evident (Figure 14). Simultaneously obvious vascular permeability changes become present, with the appearance of interfibrillar edema, in a discrete form at first (Figure 15).

The study shows that changes in endothelial cells gradually worsen, degeneration being reflected by granulovascular aspect in old age, so the some cells appear swollen at a certain moment while some others have the tendency of to detach. These structural changes are progressive and result in a gradual loss of the permeability function of the area, quantitatively and qualitatively controlled – the part served by that vessel. Histological research shows that there are larger quantities of extravasated fluid than normal, which results in increased edema, which appears more pronounced perivascularly (Figure 16).

The lumen of some vessels appears much broader, suggesting a stasis becoming more pronounced at their level. Simultaneously it is found that the interfibrillar edema is increased in the area served by the respective vessels (Figure 17).

Degenerative and alterative processes that affect the components of vascular walls do not alter all vessels at the same time, as we could observe vessels with normal structure, next to others that show advanced or very advanced alterations (Figure 18).

There are also large or even very large differences from one area to another, meaning that in some areas vascular alterations include a larger number of vessels and even seem to progress much faster. In all cases studied, the smallest vessels, with the thinnest walls, appear to be the most vulnerable. Our research shows that the alterative processes were also observed in the case of some larger caliber vessels, with thicker walls. Here, degenerative and alterative processes affect all components of the vascular wall, both the epithelial ones, namely of the endothelium, and the muscular and conjunctive ones. The loss of functionality in the case of some vessels is suggested by the tendency of thrombus organization, which tends to fill the vessel lumen, making circulation more difficult and accentuating processes of stasis and swelling. Morphological aspects suggest that skin vasculature is different, depending on the moment when the investigation is done.

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Figure 5 – The fragmentation of most elastic fibers in the superficial dermis (Orcein–Goldner’s trichrome stain, ob. ×16).

Figure 6 – Fragments of elastic fibers with low tinctorial affinity, suggesting the installation of elastolysis processes (Orcein–Goldner’s trichrome stain, ob. ×40).
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Figure 7 – Thickening of elastic fibers in the deep dermis (Orcein–Goldner’s trichrome stain, ob. ×40).

Figure 8 – Fragmented and unfragmented elastic fibers in approximately equal proportions in the deep dermis (Orcein–Goldner’s trichrome stain, ob. ×40).

Figure 9 – The generalization of the fragmentation process of elastic fibers in the deep dermis (Orcein–Goldner’s trichrome stain, ob. ×40).

Figure 10 – Gradual loss of tinctorial affinity of elastic fibers fragments (Orcein–Goldner’s trichrome stain, ob. ×40).

Figure 11 – Blood vessels, present in high number at the level of the superficial dermis (Orcein–Goldner’s trichrome stain, ob. ×16).

Figure 12 – Obvious reduction in the number of vessels in the superficial dermis (Orcein–Goldner’s trichrome stain, ob. ×40).
During the ageing process, the elastic fibers of the skin undergo degeneration and lysis, changes that have been emphasized by other studies as well [1, 3]. It should be mentioned here that the changes we have observed seem to have an irreversible character, or at least we have not observed processes that would suggest the synthesis of new fibers that might replace the ones that disappear. Elastic fibers seem to be more sensitive to processes of fibrillar lysis, as compared to collagen fibers; the lysis of elastic fibers is a slow and gradual process. Thus, after the age of 70–75 years, the process affects the vast majority of elastic fibers [8, 9].

The phenomena of degeneration, degradation, and intensification of elastolysis speed, which occur with ageing, have been discussed by other researchers as well [8, 10]. Current literature in the field includes data demonstrating that elastic fibers are thicker in the skin of elder persons as compared to skin of younger individuals and that the alterations of elastic fibers extend in the superficial and the profound dermis with ageing [4, 5, 11].
At people about the age of 70 years, fragments of elastic fibers begin to lose their tinctorial affinity for Orcein and their function is also altered. The elastolysis process seems to begin at about the same time in the superficial and in the deep dermis, although the subsequent developments seem to be slightly different [4, 9, 11]. Elastolysis is more rapid in the superficial dermis, an aspect that may be somehow associated with the fact that, in the superficial dermis, the elastic fibers are thinner than those existing in the deep dermis. The thinning of elastic fibers, their loss of verticality and rarefaction in the superficial dermis has been mentioned by other studies in the literature [3, 4]. Elastic fibers lysis has unfavorable consequences upon the structure and functionality of the skin, since these fibers are the morphological support of elasticity. The gradual disappearance of elastic fibers results in the progressive loss of skin elasticity and the appearance of characteristic clinical signs that accompany the ageing process of the skin.

Collagen fibers also undergo changes that run somewhat parallel to those affecting elastic fibers, but the speed of running and their consequences upon the structure and functionality of the skin are largely different. In the first phase, the thinnest fibers are affected. Then, with ageing, the process is extended, as demonstrated by our study, so that more and more fibers at the level of the superficial dermis undergo fragmentation processes and finally, that is after the age of 70–75 years, they are affected by lysis [5].

Superficial dermis thinning is clearly due to the quantitative reduction of collagen fibers, while the increase in density can be associated with the fact that the thinnest fibers are lysated first, so that they disappear almost entirely, the thick yet slightly larger ones remaining [5, 10]. Collagen fiber thickening occurs in the deep dermis, which gives a certain density to this layer. The fragmentation of collagen fibers is also present in the deep dermis, where a tendency of slow and gradual amplification of such processes may be observed, alongside the lysis of collagen fibers, which occurs with age. The lysis of a certain amount of fibers determines the appearance of spaces between the remaining fibers, which seem small at first but then grow in size, so that in its whole, the deep dermis seems to be made up of large collagen fibers, which are not very dense [12, 13].

Our histological study [4, 8] has demonstrated that particular aspects of collagen fibers appear in the deep dermis of some people over the age of 75–80 years. They consist of a tendency to separate the fibrils that make up collagen fibers, which in this case appear thick or very thick, though thin looking and teased, which will have repercussions on the function of these fibers. In such a situation, the dermis is far less resistant than normally. Furthermore, it seems that the process will not stop here but will continue, affecting more and more components of the dermis structure, which will have direct consequences upon the function of resistance that the dermis gives to the skin. It should be mentioned here that these last aspects have not been notified in all cases studied, but only in the case of some of the persons investigated [6, 13].

The histological investigation suggests a direct relationship between vasculature and the ageing process on the one hand, and between vasculature and the tissue served on the other hand [6, 14]. Epidermis nutrition is provided by blood vessels in the superficial dermis, and the higher the number of cell layers of the epidermis, the more demanding nutrition is, requiring a larger exchange surface. This is achieved by the presence of dermal papillae that are higher in number when the dermis has more cell layers [15].

The striking loss of vascular bed, related to age, is perceived to emphasize physiological alterations of the old skin [12, 16].

Vascular changes, with the thinning of capillaries walls and slowing of microcirculation is demonstrated by other authors [2, 16]. The changes consist in the reduction of the number and size of vessels diameter but also in alterations of vessels walls.

In the first phase alterations occur in endothelial cells, this being initially translated by granular degeneration, which becomes more evident with age. Simultaneously there are changes of vascular permeability and interfibrillar edema appear [2, 6]. Progressively the alterations of endothelial cells become more severe and degeneration is reflected by the granulo-vascular aspect; around the age of 75 years they appear swollen and tend to detach. Membrane cells also become reduced in older people, which explain vascular fragility [17, 18].

Because of progressive structural deterioration, one should mention the gradual loss of the permeability function, while the vascular stasis is suggested by the enlarged lumen of some vessels.

Not all these alternative and degenerative processes of vascular walls affect all vessels at the same time, as there are vessels with normal structure. The most vulnerable ones are the small and thin vessels [19, 20].

Decreased vascular efficiency, especially in the superficial dermis, produces a series of major effects in the epidermis, by adapting it to the efficiency of vasculature, namely by reducing the number of cell layers, i.e. by reducing the thickness [19, 21].

The most important histological change is dermo-epidermal junction flattening with a reduction of more than 50% in the number of interdigitations per unit area of the skin, between the 3rd and 9th decade [20, 22]. The progressive reduction of skin vasculature is translated morphologically through the progressive reduction of the size and number of vessels, the latter being due to alterations in vascular wall components, changes gradually leading to the cessation of the vessel functionality [15, 20]. From this point of view, all the changes appearing during the ageing process seem to run in cascade, some conditioning the appearance of others.

We might say because of our study that the starting point of skin ageing must be sought in vascular composition, of which all the other structures vitally depend. Therefore, we believe that progressive alterations in the structure and function of vessels are crucial in triggering and running all degenerative processes that accompany the ageing of the skin.

All the changes that occur in other parts of the skin are nothing but consequences of vascular alteration.
Conclusions

The histological techniques used have shown that processes of elastic fibers degeneration and lysis run faster and at higher speed as compared to the degeneration of collagen fibers and start in the superficial dermis, subsequently affecting the deep dermis as well. The consequence of connective fibers lysis in the dermis is the gradual reduction in dermis thickness as a whole, particularly through a reduction in the number and size of vessels, caused in its turn by progressive alterations of vascular wall components that go as far as the ceasing of the vessel’s function. The effects of altering the structure and functionality of vessels are critical in triggering and running all processes of degeneration and alteration that accompany skin senescence; these are changes occurring at the level of all skin components.

References


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