THE EXISTENCE OF COLLASTIN AND COLLACIN

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Biochemical and electron microscopic researches have considerably broadened our knowledge of the structure of fibrous structures. It has been discovered that an elastic fiber consists of subfibrils, existing in different combinations and embedded in a specific ground substance, the matrix [9, 13]. The subfibrils and matrix are built up of similar protein components, but acid mucopolysaccharides also enter into the composition of the matrix. Enzymes are present in the body which act upon the matrix membrane and on the fibrillary ground substance [13]. In degenerating elastic tissue Findlay [11] and Braun-Falco [7] found mucopolysaccharides, which is evidence of breakdown of the complex structures of the matrix and subfibrils.

Many problems of the pathology of fibrous structures remain unresolved. For example, the origin of the elastic-like material accumulating with age in the skin of exposed parts of the body is uncertain. Similar changes are encountered in certain other conditions. Since the time of Schmidt [16], some authors have regarded this material as elastic tissue [5, 6, 11], which is apparently confirmed by histochemical and physical investigations [7]. Many authors in the past and at the present time, however, cite no less reliable evidence that this material is a derivative of collagen [3, 13, 15]. Difficulties in the definition of elastin and collagen have led to the creation of the hypothesis that elastin may be synthesized from degradation products of collagen and that intermediate products may be formed in the tissues in the presence of disturbances of the enzyme system regulating the different aspects of fibrillogenesis and fibrillolysis which take place in certain pathological states [13].

Much of this problem might have been explained by Unna's view [18] concerning the impregnation of collagen with elastin, if they had been confirmed. In a series of skin diseases and in "senile elastosis" Unna found a modified basophilic elastic tissue, known as elacin. In these conditions the fibers became separated, swelled and sometimes broke up into droplets of clumps of matter. The degenerating collagen fibers, often breaking up into clumps, were covered by normally staining and basophilic elastic and were coverted into collastin and collacin respectively. Elastin may readily be precipitated on the collagen fibers, but a more intimate bond may be formed between these substances – the combination of molecules on the surface of the collagen. More rarely these two substances merge and mingle with each other to form a "colloid". Unna further distinguished the conversion of collagen fibers into basophilic and metabasophilic collagen (without combination with elastin).

Unna had followers, but, in the words of Sternberg [17], his ideas did not evoke a wide response, although they appeared credible. Carol (8) considered that collacin is actually basophilic collagen. If elacin merges with collagen, a homogeneous mixture, or pseudocolloid is formed. Awoki [6] writes that it is not yet known whether impregnation of collagen with elastin can take place. Hass [14], in extensive survey dealing with elastic tissue, does not fully explain Unna's ideas and states that the meaning of the changes described is unknown. A. V. Rusakov [5] does not agree with Unna's interpretation of the histological findings. Nevertheless Unna is almost the only one of the older investigators whose work is mentioned at all in modern histochemical researches [3, 13].

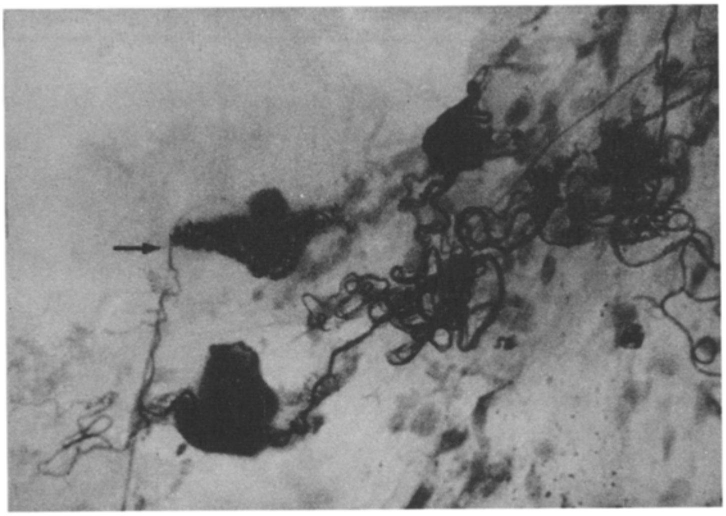

Fig. 1. Film of human loose connective tissue. Lumps of elastic tissue and, adjacent to them, elastic fibrils, here and there entwined to form tufts. The arrow points to a lump in which an area of translucency may be seen, corresponding to a collagen fiber; around it wind subfibrils. Preparation from a woman aged 23 years, dying from chronic rheumatism and heart disease. Fuchselin. Magnification 280X.

When we study films of the loose connective tissue of man and animals we are confronted by the phenomenon of the envelopment of the collagen fibers with modified elastin. The object of the present investigation was to elucidate this phenomenon.

Films were taken from the leg, the abdomen and the arm in human subjects and from various positions in animals, and stained with hematoxylin, picrofuchsin and fuchselin in various combinations, and also with polychrome methylene blue, blue polychrome, azure II, followed by subsequent treatment with a mixture of tannin and fuchsin, and with azure II-eosin.

Elastin derived from localized swellings of elastic fibers, the so-called lumps, was deposited on collagen fibers. We observed the formation of lumps in more than one third of cases (about 30 cadavers of persons aged from 23 to 75 years, dying from leukemia, tumors, contracted kidney and other diseases). Of 11 monkeys (Marcus rhesus) aged 1-2 years, sacrificed for preparation of poliomyelitis vaccine, half of which had suffered from dysentery or pneumonia, they were found in two. The lumps were particularly numerous in the connective tissue of a three year-old monkey which had died from dysentery. Lumps were found in a four year-old rabbit but were absent in a two year-old rabbit, in five dogs and in one sheep. No diseases were noted in these animals.

The lumps were distributed irregularly, in some films as many as 30-35 being present in one field of vision of the microscope with magnification 56X (diameter of the field of vision 2 mm), whereas in others only one or two could be found. The more films were studied the greater was the possiblity of finding swelling of elastic tissue in a particular individual.

The lumps arose as thickenings of the fibers, most of them consisting of bundles of fine companion fibrils and a homogeneous part, forming the groundwork of the swelling (Figs. 1 and 3, 1). Some lumps stained readily with fuchselin, just as well as the adjacent segments of the fiber from which they arose, whereas others were only weakly detected by this stain, and their homogeneous part (matrix) was almost indistinguishable from the background of the preparation, although their subfibrils were easily seen. These lumps gave off only fragments of fibers, which had lost their homogeneity and appeared as bundles of fibrils, continuous with the subfibrils of the lump. Since the formation of lumps has been regarded since the time of Schmidt's [16] research as degeneration of the fibers [6, 10, 18], but in our preparations they were found in sick monkeys* and in human patients (mainly

* We explained the swellings in the rabbit by the animal's age.

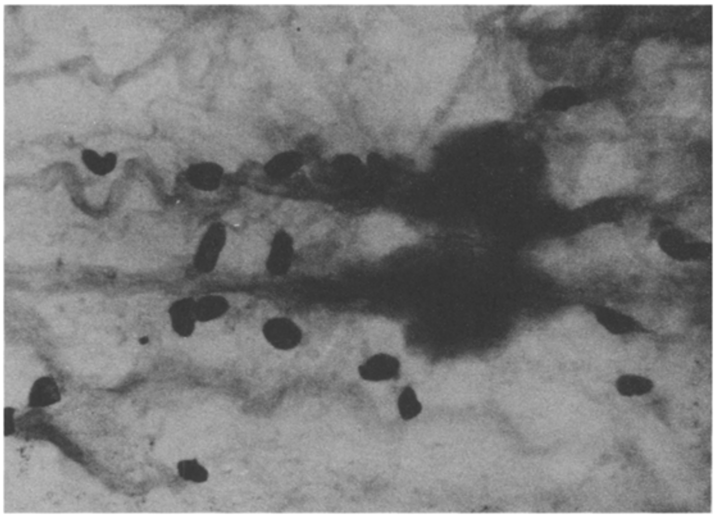

Fig. 2. Film of loose connective tissue (Macacus rhesus, aged 3 years). Basophilic homogeneous lumps. In the lumps the outlines of collagen rods can be seen. Azure II – eosin. Magnification 280×.

elderly), we understood the trend of the process to be destruction of the elastic fibers with gradual lysis of the lumps. Since, however, the lumps remained in the tissues longer than the parts of the fiber not undergoing this metamorphosis, they evidently reflected a delayed (modified) form of elastolysis.

The swellings had a tendency to be situated on collagen and elastic fibers. They could be attached to one side of the fibers, but usually they enveloped them in the form of a cuff, although in this case a very typical appearance was for this cuff to be more pronounced on one side. Staining with fuchselin showed how the subfibrils formed haphazard plexuses and entwined around the collagen fiber (Fig. 1, Fig. 3, 2-5, 8). Combinations of fuchselin with hematoxylin and picrofuchsin clearly showed up the collagen fibers but the component parts of the lumps often stained a uniform black shade. Blue polychrome and other basic aniline stains revealed the lumps (matrix) in the form of dark blue drops and cylinders, having the typical shape of the lumps or more flattened and elongated in shape, or of dark blue deposits on the collagen fibers (Fig. 3, 10-14). In this way the later stages of development of the lumps, not detected by specific staining for elastic tissue, were revealed. Voronin's hematoxylin (with ferric chloride) revealed all stages of conversion of the elastic tissue. Staining with Yasvoin' hematoxylin-picrofuchsin, in the latest stages the lumps acquired a yellowish-brown or reddish color, suggesting their relative oxyphilic properties. Such lumps could be regarded as thickenings of the collagen fibers.

Periodate–fuchsin sulfuric acid stained the swellings a red color. In view of Findlay's results [11], it may be considered that what is actually taking place in the lumps is a breakdown of the complex structure of the subfibrils and of the specific ground substance containing carbohydrates.

Staining with azure II–eosin sometimes revealed a large number of lumps, ranging from dark to light blue. Most were connected with collagen fibers. The thicker fibers were the result of the long, straight or curved shape of the drops of derivatives of the elastic substance. The thinner fibers, however, were associated with small, wedge-shaped protrusions of lumps. Some fibers within the lumps were curved in shape. The elastin drops evidently supported the affected area of the fiber and prevented it from becoming straightened during stretching of the film, and, perhaps, by retaining some degree of elasticity, returning the segment of the fiber to its original state after stretching. The surface of the collagen rods within the lumps and near to them, or less often those visibly unconnected with the lumps, often were stained a violet color with azure, which could be seen in the films as lines or interrupted marks along the edges of the fibers (Fig. 2, Fig. 3, 12). Hematoxylin also occasionally outlined the edges in this way (Fig. 3, 6). It is possible that some special substance was formed at the point where the modified elastin merged with the collagen. This was in agreement with Unna's statement [18] that the

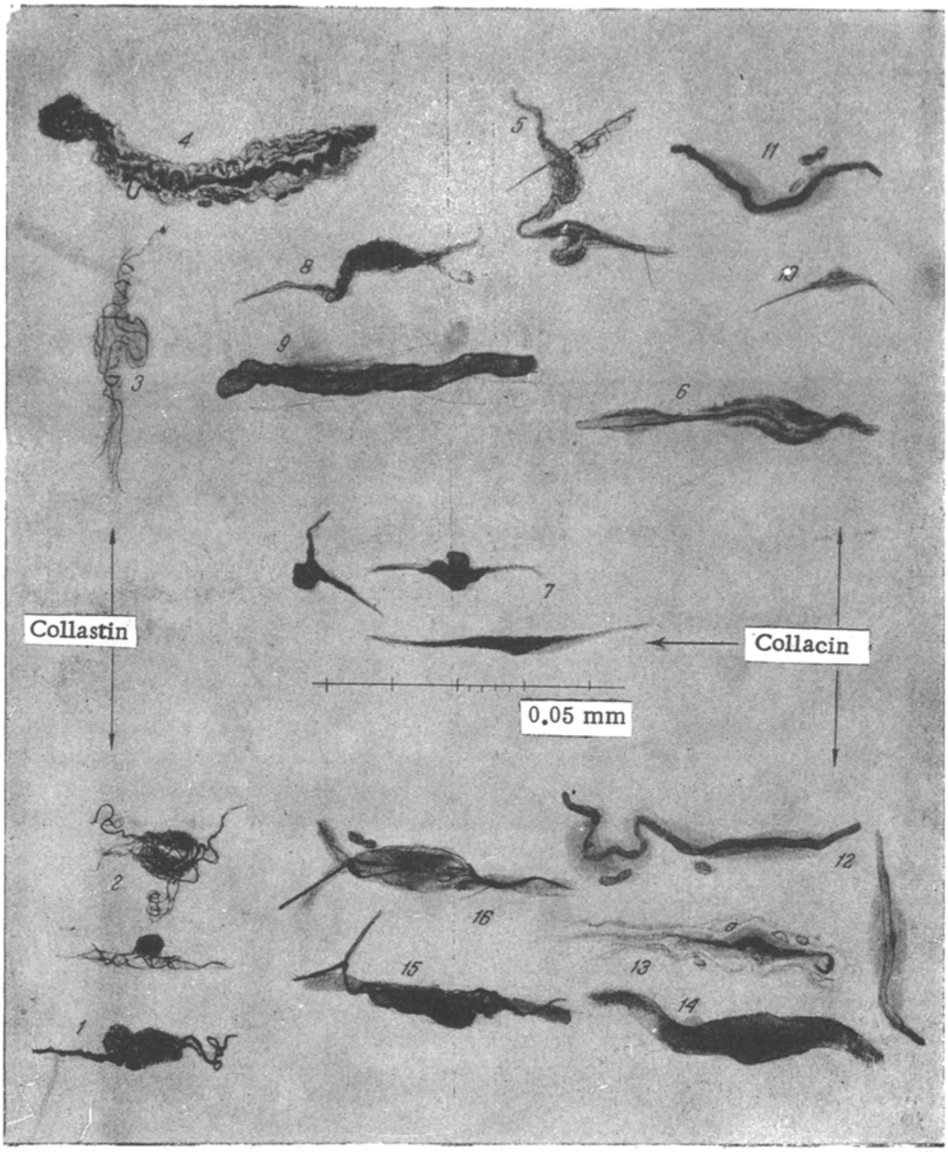

Fig. 3. Semdiagrammatic representation of the combination of elastin with collagen, reproduced by means of a drawing apparatus. Material obtained: 1, 2, 12, 13, 14, 15, 16) from human subjects; 7) from a rabbit; 3, 4, 5, 6, 8, 9, 10) from the monkey. Staining: 1-3) with fucheslin; 4-8) with fuchselin, Ehrlich's or Yasvoin's hematoxylin, picrofuchsin; 9) hemtoxylin with ferric chloride and picrofuchsin (the collagen fiber is covered with a basophilic layer, and palely stained elastic subfibrils can also be seen); 10-12) azure II–eosin; 13-14) polychrome methylene blue, tannin and fuchsin (13 – between the lumps and the adjacent collagen fibers is a translucency, probably the result of shrinkage); 15-16) fuchselin polychrome blue, tannin with fuchsin (15 – homogeneous staining with fuchselin; 16 – black subfibrils can be seen in the blue lump). For explanation see text.

molecules of the two substances were possibly combined.

In certain lumps the collagen rods were much thicker than their continuations outside the swellings. It is probable that the elastin masses, soluble with difficulty and enveloping the collagen fibers, retarded their lysis and thereby made its course irregular.

So far as changes in the collagen are concerned, we could only note that during staining with polychrome blue– tannin the fibrils of some (rare) bundles gave metachromatic staining. Sometimes such changes affected short segments in the whole thickness of the fiber. The tendency of the modified elastin to be situated on the fibrous structures may be explained by its physical properties – by the viscosity of the modified elastin. The property of elastic tissue to adhere, known since last century [1], may also be extended to collagen.

The envelopment of the collagen fibers with elastin at the stage when it stained with fuchselin corresponds to the concept of collastin, whereas their envelopment with basophilic masses corresponds to Unna's concept of collacin. Whereas the extreme stages may be strictly demarcated, however, in the intermediate stages staining with fuchselin is possible, although more weakly, as well as with basic dyes. The simultaneous staining of the subfibrils of the lump with fuchselin and of the homogeneous matrix with, for example, blue polychrome, is not, however always possible (Fig. 3, 16). Unna further pointed out that there was competition between the specific and basophilic stains. This worker doubted whether collacin must pass through a stage of collastin. It may be concluded from our investigations that this occurs very often, if not always.

The presence of small twisted fibrils and of "split" fibers in foci of degeneration of elastic tissue has been observed by many authors, but the interpretation of these changes has been made difficult by lack of knowledge of the structure of the fiber [2, 14]. M. M. Kuznets [2], for instance, describes collacin in the form of slightly basophilic collagen fibers, impregnated transversely and obliquely with very thin violet fibrils, which correspond most of all to elacin. From the study of films in which the tissue relationships are more primitive, and the changes therefore easier to explain, we are satisfied that the thin, twisted fibrils which are often observed in foci of "basophilic degeneration of the skin" are subfibrils.

From the results obtained certain suggestions regarding the changes in the skin may be made. The compact texture of the dermis must account for the more uniform distribution of the degenerating elastin, for the round lumps cannot move amid the intertwined collagen fibers. In certain diseases, moreover, the swellings of the elastic tissue may be more diffuse and their absorption even slower as a result of disturbances of the enzyme systems [13], of a decrease in the sensitivity of elastin to the action of elastase [12] and of the lesser degree of accessiblity of the fibers of the dermis to enzymes in general, by comparison with the fibers of loose connective tissue.

The fact that carbohydrates are found in degenerating elastic tissue in chronic pathological conditions such as senile elastosis, elastic pseudoxanthoma and colloid degeneration of the skin [7, 11] and in vessels of changeable caliber [4], taken together with our findings, suggests that the mucopolysaccharides causing the basophilia of pathological elastic tissue may be detected when destruction of the elastic tissue is retarded and that the discovery of elacin may indicate an abnormality of elastolysis, consisting of delayed lysis of elastomycin.

While, therefore not denying changes in collagen, including those of elastic type, we must support the correctness of the view that collagen is regularly enveloped with modified elastin, a process which occurs when elastolysis is retarded.

SUMMARY

The author studied film preparations of the loose connective tissue in man and animals suffering from various diseases. In many cases focal swellings of the lumps were detected. Their numbers increased with the age of the individual and had less to do with the disease itself. Homogenous basophile matrix part and entangled subfibrils could be identified in the lumps. The lumps had a tendency to place themselves on the collagenous fibers. In earlier stages of their evolution, fuchselin showed the presence of entangled fine elastic subfibrils around the collagenous fibers, which corresponds to Unna's conception of collastin. In later stages of evolution, only the homogeneous basophile masses of elacin are seen around the fibers. These pictures correspond to the conception of collacin. The fact of regular enveloping of fibers with dissolving elastin must be taken into account when studying the morphology of senile elastosis and certain other diseases.

LITERATURE CITED

1. M. Gardner, The Histogenesis and Structure of Elastic Tissue [in Russian] Dissertation. (Moscow, 1898).
2. M. M. Kuznets, in: Old Age [in Russian] (Kiev, 1940) p. 117.
3. U. F. Lever, Histopathology of the Skin [in Russian] (Moscow, 1958) p. 174.
4. G. V. Orlovskaya, Morphological Features due to Age in the Skin of the Face. Candidate dissertation [in Russian] (Moscow, 1947).
5. A. V. Rusakov, The Physiology and Pathology of Certain Tissues of the Internal Environment. Incomplete Desmogenesis [in Russian] (Moscow, 1954) p. 35.
6. T. Awoki, Arch. path. Anat. 255, 709 (1925).
7. O. Braun-Falco and K. Salfeld, Dermat. Wschr. 135, 369 (1957).
8. W. L. L. Carol, Acta dermat.-venereol. 3, 263 (1922).
9. N. Dettmer, Z. Zellforsch. u. mikr. Anat. 45, 265 (1956).
10. I. Ejiri, Zbl. Haut. Geschlechtskr. 55, 428 (1937).
11. G. H. Findlay, Brit. J. Dermat. 66, 16 (1954).
12. D. Hall, in: Council for International Organzation of Medical Sciences. Connective Tissue; Symposium (Oxford, 1957) p. 238.
13. D. Hall, et al., J. Gerontol. 10, 388 (1955).
14. G. M. Hass, Arch Path. 27, 334 (1939).
15. J. Kyrle, Vorlesungen über Histo-Biologie der menschlichen Haut und ihrer Erkrankungen (Vienna-Berlin, 1925), Vol. 1.
16. M. B. Schmidt, Arch. path. Anat. 125, 239 (1891).
17. C. Sternberg, Arch. path. Anat. 254, 656 (1925).
18. P. G. Unna, Monatsh. prakt. Dermat. 19, 397, 465 (1894).