their size, which correlates also with their functional activity. Further research in this direction must evidently yield fresh data on the structural and functional states of mitochondria.

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STRUCTURE AND PROPERTIES OF TENDON COLLAGEN COMPLEX DURING DISORGANIZATION OF THE GROUND SUBSTANCE OF CONNECTIVE TISSUE

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KEY WORDS: carbohydrate-protein complexes; connective tissue.

An important place in human pathology is occupied by systemic diseases of connective tissue [4, 9, 11], accompanied by disorganization of the carbohydrate–protein complexes of the ground substance of connective tissue as a result of activation of mucolytic and proteolytic enzymes [5]. Under these circumstances the collagen fibers undergo essential changes [8, 11]. Analysis of the structure and properties of the collagen skeleton during enzymic disorganization of ground substance is of great interest for the closer study of the mechanisms of development of pathological changes in connective tissues affected by systemic diseases. On the basis of the facts indicated above it was decided to study the structure and properties of the collagen complex of tendons during enzymic disorganization of the ground substance.

EXPERIMENTAL METHOD

Achilles' tendons from persons aged 25-50 years, dying from trauma, were used as the test object. The material was obtained at autopsy within 24 h after death. Treatment with the enzyme amyloryzine was carried out for 6-12-24 h at 37°C, with an enzyme:substrate ratio of 1:25. A solution of the enzyme was made up in phosphate buffer, pH 5.6. Amyloryzine has no collagenase activity and is characterized by amylolytic and by a low level of proteolytic activity. The specimens of tendon also were treated with water for 24 h. The original specimens of tendon, and material treated with the enzyme or water, were investigated by scanning and transmission electron microscopy.

The physicochemical investigations of the enzyme-treated tendon were undertaken 24 h after the beginning of incubation. The glycosaminoglycan content was determined from the quantity of amino sugars [1]. Stretch diagrams of the specimens were studied in a medium of physiological saline on an elastodynamometer [10]. The temperature of hydrothermic contraction of the tendons also was determined on the same instrument. The modulus of elasticity was calculated from the tangent of the angle of slope of the rectilinear region of the curves. The contraction temperature of the tendons when heated in a dry state (20-220°C) was recorded on the apparatus described by Kaimin' [6]. The total moisture content and the quantity of hydration-bound water were determined by Fischer's method [7].

EXPERIMENTAL RESULTS

Fibrous connective tissue of normal tendon is a complex of collagen fibers, highly organized and combined into a single biological structure. The largest collagen fibers have a mutually parallel orientation, coinciding with the direction of the main mechanical stresses to which the tendon is subjected (Fig. 1). At the same time, the tendon also has a developed

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Fig. 1

Fig. 2

Fig. 1. Spatial orientation of tendon collagen fibers, 3200 X. Scanning electron microscopy.

Fig. 2. Structure of tendon collagen fibers in section, 1600 X. Scanning electron microscopy.

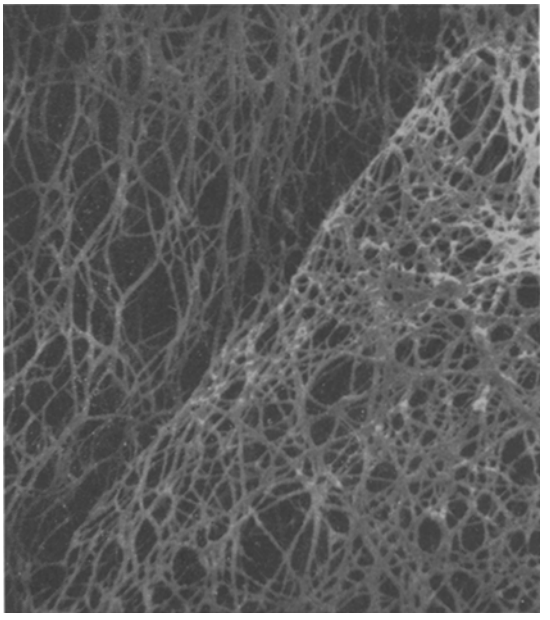

Fig. 3. Structure of collagen fibers after enzyme treatment, 3200 X. Scanning electron microscopy.

system of fibrous elements which perform an integrative function, binding the fibrils and collagen fibers in the transverse direction. In thick sections the structure of collagen fibers appears very compact because of the dense packing of the fibrils and filling of the spaces between them with ground substance (Fig. 2). The fibrils vary in diameter and have cross-striation with a period 60-70 nm in length. On treatment of the tendon with enzyme, the microstructure of the collagen fibers and of the tendon as a whole undergoes considerable changes. In the initial stage of the changes some increase in volume of the ground substance is observed, and it becomes an amorphous gelatinous mass, enveloping the fibrils. This increase was evidently due to its swelling because of decomposition of carbohydrate–protein complexes and addition of water by the free groups of the resulting products.

Meanwhile, the structure of the collagen complex of the tendons was modified: separation of the layer of collagen fibers into fibers of smaller diameter, consisting of bundles of fibrils and separate fibrils. After 24 h, where the collagen

TABLE 1. Results of Chemical and Physical Investigations of Various Tendon Preparations

Preparations studied	Hexosamine, %		Water, %		Concentration temperature, °C		Modulus of elasticity, $E \cdot 10^7$ dynes/cm^2	Residual deformation, %
	content	decrease	total	bound	water	in dry state		
Achilles' tendons treated with water	0,55	—	63,7	36,1	63,0	200,0	13,0	8,0
	0,42	23,6	67,6	36,2	63	192	8,3	12
Achilles' tendons treated with amyloryzine	0,10	81,8	69,3	37,6	59	175	4,3	35

fibers had been, there remained a network consisting of small communicating fibers of varied diameter (Fig. 3). Under these circumstances about 80% of the acid glycosaminoglycans was removed from the tendon (Table 1). Only covalently bonded glycosaminoglycans remained in the preparations, as shown by their low hexosamine content [12]. No significant changes were found in the ultrastructure of the collagen fibrils. In some cases, however, the possibility of their longitudinal splitting could not be completely ruled out. It can be postulated on the basis of existing views [2] that removal of noncovalently bonded acid glycosaminoglycans from the collagen complex removes the screening of the positively charged groups in collagen, leading to separation of the fibrils. Treatment of the tendons with water led to removal of a little more than 20% of the glycosaminoglycans (Table 1),and this was accompanied by much less conspicuous changes in the microstructure of the collagen fibers. Despite the presence of a highly specialized system of connecting fibers, which serves to integrate the fibrous structures of the tendon, the main role in stabilization of the microstructure of the collagen complex is thus played by the ground substance. A special feature of the structural conversion in collagen fibers on disorganization of the ground substance is the formation of a net-like structure containing numerous macropores. These structural changes in the collagen fibers may facilitate their penetration by water, blood plasma proteins, and other products formed in the course of the pathological process. Changes in the structure and architectonics of the collagen complex are accompanied by a decrease in the modulus of elasticity and by some increase in residual deformation of the tendon (Table 1). The collagen skeleton can no longer restore its original dimensions when the action of an external force ceases. Conversion of the structure of the collagen fibers into a net-like formation increases the mobility of the structural components of the collagen complex, which provides a good explanation of the changes observed in the rheological properties of the tendon. Disorganization of the ground substance was accompanied by disturbance of hydration of the collagen complex. An increase in the quantity of both hydration-bound and total water was observed (Table 1). The increase in the water content of the collagen complex took place despite substantial destruction of the carbohydrate–protein complexes capable of binding and retaining a large quantity of water [3]. The formation of macropores filled with water in the system evidently masks the effect of removal of the ground substance as hydrophilic carrier. On the basis of the results described above it can be postulated that the mechanism of swelling of collagen fibers on disorganization of the ground substance is complex. In the initial stages addition of water takes place through the unblocking of some of the polar groups of the collagen and of proteoglycans. As disorganization continues and the ground substance is removed, swelling and an increase in volume of the collagen fibers may take place mainly as a result of structural conversions of the system. The temperature of hydrothermic contraction of tendons after enzyme treatment falls by 4°C and the contraction temperature of the specimen during heating in the dry state by 25°C. The lowering of the temperature of the phase transition is evidence of a disturbance of the structural stability of collagen, thereby confirming existing data on the participation of glycosaminoglycans in stabilizing the molecular structure of collagen [13]. At the same time it had no appreciable effect on the ultrastructure of the collagen fibrils.

This investigation thus showed that enzymic disorganization of the carbohydrate–protein complexes of the ground substance of connective tissue is accompanied by considerable changes in the microstructure of collagen fibers. The fibrous connective tissue loses its organ-specific structural and architectonic features. Structural conversions of collagen fibers have a significant effect on the physicochemical and rheologic properties of the collagen complex.

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HISTOMORPHOLOGICAL REACTION OF THE TEETH AND PARODONTIUM TO DRILLING BENEATH A METAL–CERAMIC CROWN

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KEY WORDS: drilling of teeth; metal–ceramic crowns; histomorphological changes.

Metal–ceramic crowns are the most functional, esthetic, biologically inert, yet sufficiently strong type of fixed dental prostheses [1, 3, 5, 7]. However, when the tooth is drilled under this type of artificial crown, there is much more abrasion of the hard tissues than under a metal crown.

No information could be found in the accessible literature on the histochemical and neurohistological reaction of the teeth and parodontium to drilling beneath a metal–ceramic crown, despite its great scientific and practical importance. The aim of the investigation described below was to study this problem.

EXPERIMENTAL METHOD

Experiments were carried out on 36 mongrel dogs with an intact maxillodental system and aged between 10 months and 2 years. In each dog three teeth were drilled with an electric drill, the bit of which revolved at a speed of 10,000 rpm, beneath metal–ceramic crowns: with no projection, with a cervical projection on the vestibular and occlusal surfaces, and with a circular projection. The hard tissues of the teeth were drilled under morphine-thiopental anesthesia, using the standard technique and cooling the tooth with water while it was being drilled. The animals were killed under morphine-thiopental anesthesia by exsanguination through the femoral artery immediately after the operation and also after intervals of 1 h and 1, 3, 7, 14, 21, 28, and 35 days. The teeth and the parodontal tissues were fixed in 10% neutral formalin solution (18 dogs) and in Shabadash's fluid (18 dogs). Fragments of the jaw with the teeth were decalcified in a 25% solution of Trilon B. The teeth and parodontal tissues of 10 dogs not undergoing the procedures served as the control. Sections through the teeth and parodontal tissues were stained with hematoxylin and eosin and by Van Gieson's method and impregnated with silver nitrate by the Bielschowsky–Gros and Rasskazova methods. Sections through the pulp were stained for RNA by Brachet's method, for glycogen by Shabadash's method, and for acid mucopolysaccharides by Steedman's and Hale's methods. The enzyme-chemical control consisted of incubation of the sections with bovine ribonuclease, amylase, and bacterial hyaluronidase.

EXPERIMENTAL RESULTS

Unlike the teeth of the control dogs, many of the small blood vessels of the capillary and aterial network of the crown pulp of the drilled teeth in dogs killed immediately after drilling were sharply dilated and congested with blood. Intensification of the pattern of the precapillary and capillary network of the odontoblastic and subodontoblastic layers was observed, and it was denser because of dilatation of the functioning vessels and filling of the reserve vessels with blood. The nerve endings and fibers of the subodontoblastic plexus, preterminal and terminal branches of the perivascular nerve fibers of the crown pulp of the tooth, and axons of most of the large-caliber myelinated and unmyelinated nerve fibers of the crown and central layers of the root pulp were thickened, hyperargentophilic, irregular in outline, and possessed many varicose expansions, circular, oval, or fusiform in shape. Morphological changes affected both the freely lying nerve fibers and those contained in bundles and trunks. In the same bundle some axons were in a state of irritation, others preserved their normal structure. This confirms data in the literature on the existence of more or less resistant nerve fibers [2, 4, 6]. The terminal branches and axons of some nerve fibers in the periapical region of the parodontium of the alvelus and of the alveolar septa were uniformly and strongly impregnated with silver, with uneven outlines and with numerous varicose expansions (Fig. 1). The intensity of staining of both nucleolar and cytoplasmic RNA and of glycogen was appreciably

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