from falling toward the root of the tongue. It ensures that the basic flow of the food passes around the base toward the molar teeth. The large conical papillae may also be mechanoreceptor formations. The long and delicate processes of the digitate papillae evidently help to direct the flow of already masticated food into the pharynx. This explanation is supported by the presence of numerous "grooves," formed by digitate papillae, running toward the circumvallate papilla. Evaluation of the taste of the food in the course of swallowing is a function of the chemoreceptors of the circumvallate papilla.

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ROLE OF CARBOHYDRATE – PROTEIN COMPLEXES IN THE ORGANIZATION OF THE MICROSTRUCTURE OF FIBROUS CONNECTIVE TISSUE

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Samples of tendon and fascia, treated with enzymes possessing amylolytic and proteolytic activity (protorysin, trypsin) were investigated by scanning electron microscopy. Destruction of carbohydrate–protein complexes leads to disappearance of the characteristic morphological features of the collagen fibers. Construction of the collagen fiber was shown to be based on a network of thin anastomosing fibrils, forming a carcass connected with the carbohydrate–protein matrix. It is argued that the reticular structure is a general principle in the structural organization of fibrous connective tissues.

KEY WORDS: Scanning electron microscopy; connective tissue; collagen fiber; protorysin; trypsin.

The use of modern methods of morphological analysis combined with enzymic action on complexes of biopolymers has provided fresh opportunities for the study of their role in the structural organization and functions of different tissues. A current direction of such research is the study of the role of carbohydrate–protein complexes in the structural organization of fibrous connective tissue. This is due to the participation of the substances mentioned above in pathological processes connected with lesions of the connective tissue, and also their influence on some important biomechanical properties of the fibrous carcass [7, 10, 12]. The presence of protein complexes of glycosaminoglycans and glycoproteins in the collagen fiber has been established and evidence has been obtained that these complexes participate in fibrillogenesis and are concerned in the orderly union of fibrils into bundles [8, 11, 13].

The object of the present investigation was to study the role of carbohydrate–protein complexes in the organization of the microstructure of fibrous connective tissue. For this purpose a morphological analysis was made of the connective tissue of fascia and tendon after treatment with enzymes decomposing carbohydrate–protein complexes.

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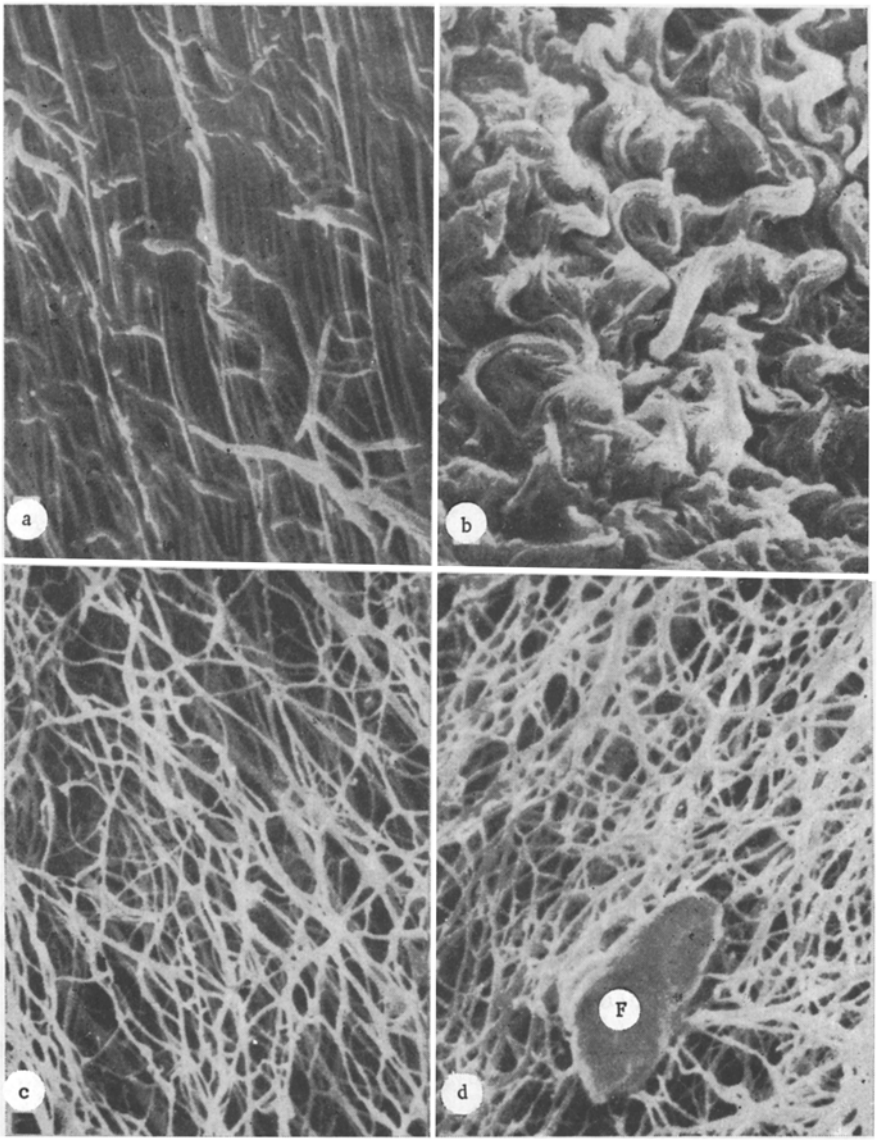

Fig. 1. Structure of fibrous connective tissue of tendon and fascia before and after treatment with protorysin for 18 h: a, c) tendon before and after treatment with protorysin, respectively; b, d) fascia before and after treatment with protorysin, respectively. F) Fibroblast: a) 4120 ×; b) 1050 ×; c, d) 3500 ×.

EXPERIMENTAL METHOD

Ten samples of tendon and fascia from human muscle from subjects aged 20-50 years served as the test object. The material was obtained during autopsy within 24 h after death which occurred from trauma. Both fixed material and samples treated with the enzymes protorysin and trypsin without preliminary fixation were used. For fixation of the samples of tendon and fascia they were immersed in a 10% solution of formalin, pH 7.0, for three days. Unfixed material was treated with protorysin and trypsin. Protorysin is an enzyme with amylolytic but only negligible proteolytic activity [4, 5]. It has neither collagenase nor elastase activity. Protorysin has been successfully used in morphological investigations to remove the ground substance of connective tissue [3]. Samples of fascia and tendon were placed in test tubes containing phosphate buffer, pH 5.5. Protorysin was added to the medium in a ratio of enzyme to substrate of 1 : 25, after which the material was incubated for 18 h at 37°C. When trypsin was used the ratio of enzyme to substrate was 1 : 10, in medium with pH 7.5-7.7. The material was processed for 10 h at 38°C. After enzyme treatment the samples were washed with water for 30 min, then placed in liquid nitrogen and dried in vacuo. The fixed material and samples treated with enzymes were sprayed with copper and examined in the Stereoscan 4S-10 scanning electron microscope. Before spraying, the fixed material was dehydrated in alcohols.

EXPERIMENTAL RESULTS

The structural organization of the fibrous connective tissue of the tendon and fascia has certain specific distinguishing features. In tendon it is a system of parallel collagen fibers which are organized into cylindrical bundles. The fibers anastomose with each other; as a result they acquire structural integration and their characteristic architectonics is maintained (Fig. 1a). The structure of the fibrous connective tissue of the fascia is characterized by a complex plexus of flattened, undulating fibers. The fibers communicate with each other by means of thin fibrils, which have the appearance of twigs or bridges (Fig. 1b).

Treatment of the tendon and fascia with protorysin led to disappearance of the collagen fibers (Fig. 1c, d). The connective tissue lost its specific features, which were due to the different morphological characteristics and architectonics of the collagen fibers. Instead of collagen fibers a dense homogeneous network of anastomosing fibrils 0.1-0.3 μ thick could be seen. After treatment with trypsin, besides preservation of the usual morphological picture of the collagen fiber, its internal reticular structure was revealed in the form of a thinly fibrous carcass.

The results of the investigation thus show that carbohydrate–protein complexes play an exceptionally important role in the formation of the microstructure of the collagen fiber, determining its configuration, dimensions, and spatial orientation. On the basis of these results ideas on the collagen fiber as a formation consisting of bundles of fibrils running predominantly in the same direction must be revised. According to the observations now made, the structure of the collagen fiber is a network of anastomosing fibrils 0.1-0.3 μ thick, which form a carcass connected with a carbohydrate–protein matrix. In their size and their characteristic organization in a network the fibrils correspond to reticulin fibrils. On the basis of the results of this investigation it can be postulated that a network of fibrils 0.1-0.3 μ in diameter is a unified component in the structural organization of connective tissue, on the basis of which collagen fibers are formed. The principle of reticular structure is also repeated at a higher level of structural organization of fibrous connective tissue. This is shown by the numerous anastomoses between fibers in both fascia and tendon. Previous investigations [9] showed that the fibrous connective tissue of the endomysium and inner perimysium of skeletal muscle is also a complex network of anastomosing fibers. Together with collagen fibers, elastic fibers also are organized into reticular structures [7]. Networks with different architectonics form the connective-tissue fibers of skin, bone, and the large arteries [1-3].

It can thus be concluded from the results of these investigations and also from data in the literature that the reticular structure is a general principle of the structural organization of fibrous connective tissue. It enables connective-tissue fibers to be unified into a single functional system. Depending on the functions peculiar to each organ, the above-mentioned principle of structural organization of connective tissue assumes specific morphological forms.

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