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Morphology

# Homology in Structural and Functional Organization of the Fibrous Framework of the Derma and Paraneural Connective Tissue Components

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## Abstract

***Background***: The recent increase in hostilities throughout the globe is one of the main factors that damage both the skin and peripheral nerves (PN), mainly of the upper and lower extremities. The duration of treatment of the injured limb and the rate of recovery of its functions are directly related to the structural integrity of the connective tissue (CT) sheath apparatus of the PN, which further actualizes the study of its micromorphological components. The structural integrity of the peripheral nerve includes not only the preservation of its conductive component but also the membranes surrounding it, providing complete morphofunctional unity. Thus, the study of the features of their tissue organization, vascularization and innervation, with the obligatory comparison of data with homologous tissue of different topography and possible extrapolation of data, determined the purpose of this work. The aim of this study was to analyze the dynamics of changes in the fibrous components of the paraneurium and dermis in onto- and phylogenesis under the influence of various factors and to compare the data obtained for their further extrapolation.

***Methods and Results:*** The study was conducted on mature male Wistar rats under standard vivarium conditions. The material for the study was the CT sheaths of the PN of the extremities and skin areas, 1x1 cm in size, to the depth of the subcutaneous fascia of animals. The cadaver material was taken on Days 1, 3, 7, 10, and 28 of ontogeny. The resulting biomaterial was fixed in a 10% buffered neutral formalin solution and embedded in paraffin, according to the standard method. Microtomized, the resulting histological sections were stained with hematoxylin and eosin, according to the method of Mallory and Van Gieson. A comprehensive morphological study was performed using light and electron microscopy. The study revealed the presence of two stages in the ontogeny of the CT, forming the dermis of the skin and paraneural CT structures of PN. In the first stage, structural elements are formed, considering the topography of the CT; in the second, they are differentiated, leading to a qualitative-quantitative transformation due to the appearance of a number of additional functions, an active period of body growth, and taking into account the action of various environmental factors. At earlier stages of ontogenesis, thin, flattened fibrous structures without a clear organization are observed in the field of view in the dermis of the skin. In the paraneurium, the components that form it are not sufficiently expressed. At later periods, the shape and architectonics of the fibrous component change, structurization and an increase in the thickness of the fibers occur and pronounced heterogeneity of the cellular component is observed.(**International Journal of Biomedicine. 2022;12(4):644-647.**).

**Keywords**: skin dermis • connective tissue • collagen fibers • fibroblasts • regeneration • paraneural membrane

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## Abbreviations

**CT**, connective tissue; **PN**, peripheral nerves.

## Introduction

Despite significant achievements in practical neurosurgery in performing reconstructive operations on the trunks of peripheral neurons, there are still unresolved issues regarding the need to perform restorative manipulations on the connective tissue (CT) membranes surrounding the bundles of nerve fibers.[1-3] Endo-, peri-, epi- and paraneural CT sheaths of peripheral nerves (PN), having one fundamental morphological basis, perform different functions due to a slightly different list of structural elements and topographic features.[4,5]

In domestic and foreign literature, sufficient data are presented on the structure and functions of the epi-, peri- and endoneural membranes. At the same time, data on the morphological and functional organization of the paraneural membrane are scattered and incomplete.[6,7] One of the first to study the structure of the paraneural membrane and determine its role in the peripheral nerve (sciatic nerve) was the domestic morphologist V. S. Polsky (1991). After a quarter of a century, another domestic morphologist, M. A. Zatolokina (2017), studied the paraneural apparatus of the PN of the branches of the brachial plexus in a phylogenetic aspect. In the works of foreign scientists, such as J. D. Vlok (1997), Andersen et al. (2012), O. Choquet et al. (2012), and M. Karmakar et al. (2013), data were developed that allowed the functional role of paraneural CT structures to be somewhat specified. Regarding the skin, it should be noted that external influences and any damage to internal organs (in this case, the PN of the limbs) are associated with the destruction of the constituent elements of the skin. In this regard, the study of the morphology of the constituent elements of the skin in the norm (ontogenesis) and under the influence of various factors (humid environment, physical activity) does not lose its relevance at the present time.[8-10]

The main working hypothesis of this work was that some homology is present in the structure of the fibrous base of the paraneural membrane of the peripheral nerve and the dermis of the skin. Considering the almost identical structural set of paraneurium and dermis components, the goal of this work was formulated: to analyze the dynamics of changes in the structural components of paraneurium and dermis in ontogenesis and phylogenesis under the influence of various factors and to compare the data obtained for their further extrapolation.

## Materials and Methods

In vivo experiments were carried out in accordance with the legislation of the Russian Federation, in strict compliance with the European Convention for the protection of animals used for experimental and other purposes (Strasbourg, France, 1986), the provisions of Directive 2010/63/EU of the European Parliament and the Council of the European Union of 22 September 2010 on the protection of animals used for scientific purposes (Article 27), and approved by the Regional Ethics Committee of Kursk State Medical University.

The study was conducted on mature male Wistar rats under standard vivarium conditions. The material for the study was the CT sheaths of the PN of the extremities and skin areas, 1x1 cm in size, to the depth of the subcutaneous fascia of animals. The cadaver material was taken on Days 1, 3, 7, 10, and 28 of ontogeny. The resulting biomaterial was fixed in a 10% buffered neutral formalin solution and embedded in paraffin, according to the standard method. Microtomized, the resulting histological sections were stained with hematoxylin and eosin, according to the method of Mallory and Van Gieson. For scanning electron microscopy (SEM), the material, after fixation, was dehydrated in a frozen state in alcohols of increasing concentrations and mounted on a special aluminum table with conductive carbon glue, sputtered with a platinum-palladium alloy in a Quorum Q150TS sputtering unit (Germany). Next, a comprehensive morphological study was performed using light and electron microscopy (SEM - S 3400N, Hitachi, Japan). The dynamics of changes in morphometry data were assessed by such indicators as the CT density coefficient (CTDC) and the heterogeneity of the cell population. CTDC was calculated as the ratio of the area occupied by the fibers (A-f, %) to the area of interfiber gaps (A-ig, %). A-f and A-ig were determined in 30 fields of view on digitized micrographs after their geometric and optical calibration using the ImageJ 14.7a program. The heterogeneity of the cell population was assessed by karyological identification of cells visualized between the structures of the fibrous component—resident and non-resident cells were counted in 10 non-overlapping fields of view per 100 cells.

Statistical analysis was performed using using the program Statistika 10.0 (StatSoft). The mean (M) and standard error of the mean (SEM) were calculated. Differences of continuous variables were tested by the Mann-Whitney U-test. A probability value of $P \leq 0.05$ was considered statistically significant.

## Results and Discussion

The main working hypothesis of this work was that homology is present in the structure of the fibrous base of the skin dermis and the paraneural apparatus of the PN. At the heart of two topographically different parts of a living organism, the skin and the paraneural membrane, is the CT, and the source of development in embryogenesis is the mesenchyme. Given this fact, it is quite logical to assume that these topographic areas are similar not only in the structure of the CT, but also in the performance of the same morphologically substantiated functions under the condition of the same external factors.

The first stage of the study, including an examination of the features of the structural organization of the fibrous component of the paraneural membrane and the dermis of the skin in laboratory animals (rats at different periods of ontogenesis), made it possible to state a similar vector direction in the dynamics of the development of the CT that forms the above indicated topographic areas.

The paraneurium of the PN of the extremities is a complex of CT, vascular, and nervous structures located between the epineurium and the fascia of the adjacent muscles and having a close morphofunctional relationship with the nerve trunk. The CT basis of the paraneurium includes its own fascial sheath and fibrillar cords extending from it, separating the fascial-cellular spaces (filled with adipose tissue) and connecting the paraneural apparatus with the fascia and interfascial spaces of the adjacent muscles. In the early stages of postnatal ontogenesis (1-3 days after birth), the fibrillar structures of the paraneurium are very poorly developed. Fascial sheath is formed by several CT fibers located relative to each other loosely and not ordered. Fibrillar strands are not identified, fascial-cellular spaces are just beginning to form, and white adipose tissue is visualized in them with an unexpressed lobular organization.

In sections of the dermis, the fibrous component looks like thin plates with a large number of branches. The high density of the cellular component is noteworthy in comparison with the paraneural structures, where it is insignificant. The thickness of the fibrils varied from 28 to 32±0.1 nm, and the collagen fibers formed from them were 5.2±0.3 μm.

On Days 7-10 of ontogenesis, a complication of the structure of the paraneural apparatus was observed, manifested in a pronounced morphological representation of all its constituent components, as well as the structural organization of the dermis. The thickness of the fascial sheath was 2.47±0.11 μm, and that of the outgoing CT slings was 0.49±0.01 μm. The fibrous component was represented by wavy, transversely striated collagen fibers, which, when stained by the Mallory method, had a bright blue color. It should be noted that the change in the shape of collagen structures on longitudinal sections from lamellar to fibrous, on transverse sections, the shape was rounded. At the same time, the thickness of collagen fibrils was 38±1.8 nm; they were located in parallel. The cellular component was heteromorphic; several differons were identified by karyological features. The density of cells was 1.5 to 2 times higher than at the previous stage of ontogenesis.

In the late stages of ontogenesis (puberty), in the organization of both the fibrous skeleton of the dermis and the paraneurium, the rhomboid type in the arrangement of fibrous structures predominates. Thickening and spiralization of collagen fibers were observed in the dermis. On transverse sections, their shape varies from flattened to rounded. The diameter of fibrils forming collagen fibers ranged from 70 nm to 90 nm. In the structural organization of the paraneurium, a 1.7-fold thickening of its own fascial sheath and CT slings was observed. The fibers visualized in them have a spiral course, which smoothly turns into lines. Between the slings are well-defined lobules of adipose.

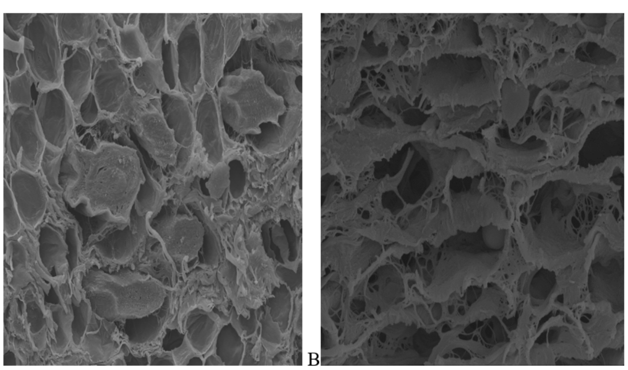
A B

***Fig. 1.*** *Micrograph of connective tissue (collagen fibers): A – Paraneuria; B- In the dermis of the skin. Magnification x1200. SEM.*

Thus, in the ontogeny of the CT structures of the paraneurium and the dermis of the skin, their two-stage activity was observed. In the first stage, the necessary list of structural elements was formed; in the second stage - their differentiation, which consists of a qualitative and quantitative transformation due to the appearance of several additional functions, an active period of body growth, and taking into account the action of various environmental factors.

**In conclusion**, the study revealed the presence of two stages in the ontogeny of the CT, forming the dermis of the skin and paraneural CT structures of PN. In the first stage, structural elements are formed, considering the topography of the CT; in the second, they are differentiated, leading to a qualitative-quantitative transformation due to the appearance of a number of additional functions, an active period of body growth, and taking into account the action of various environmental factors. At earlier stages of ontogenesis, thin, flattened fibrous structures without a clear organization are observed in the field of view in the dermis of the skin. In the paraneurium, the components that form it are not sufficiently expressed. At later periods, the shape and architectonics of the fibrous component change, structurization and an increase in the thickness of the fibers occur and pronounced heterogeneity of the cellular component is observed.

The data obtained during the work fully comply with the Lesgaft law: the form and functions are the same, which allows one to transfer the observed changes in the CT of the dermis in conditions of damage or inflammation to the paraneural CT structures. Also, it is possible to predetermine the dynamics of the course of pathological changes and disorders in the future of the functions of those structures for which the paraneurium components are fundamental (the conductive component of the PN in this case). Homologous changes in the fibrous skeleton of the dermis and paraneurium observed during ontogeny make it possible, with a certain degree of probability, to extrapolate the obtained data on changes in the dermis to the paraneural structures of the PN of the extremities.

## Competing Interests

The authors declare that they have no competing interests.

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