THE RELATIONSHIP BETWEEN CERTAIN PHYSIOLOGICAL AND HISTOMORPHOLOGICAL CHANGES IN THE NEUROMUSCULAR APPARATUS OF ANIMALS ASSOCIATED WITH AGING

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With aging of the organism, along with changes in the functional properties of its individual systems, there also occur structural changes in the tissues of these systems. It remains unknown whether these processes develop in parallel, or whether one of them preceeds another, causing a decrease in the functional capacities due to disturbances in structure, or causing dystrophic structural changes owing to a reduction in functioning.

In the majority of cases, a study of morphological changes is done differently from studies of the determination of the functional properties of tissues. The chronological comparison which is then done cannot be convincing, since at the very same age the degree of development of structural and functional changes can be different.

Studying the physiological properties of muscles during aging of the organism, we come up against particularly serious difficulties. The response of muscles to one or another form of stimulus depends on the condition of the muscle fibers, the nerve conductors, and their endings. An important role is also played by the condition of the center, but in this work we will only consider the muscle and its innervating nerve.

Our goal was to characterize the reciprocal relation and dependency of structural aging and the appearance of features in the course of the reactions, as well as changes in the basic indices for the excitability of muscles during aging.

It was shown earlier [3] that, in the very same muscle, by far not all the muscle fibers undergo the same structural changes during aging. In some, one observes all the signs of so-called senile atrophy [1]; in others – only some of these signs, for example, nuclear multiplication; and finally, in the third group, destructive changes do not arise. Therefore, the muscle, as an intact organ, consists, during the aging process, of muscle fibers which are not uniform in structure, and thus, not uniform in biological activity. This fact complicates a determination of the role of structural changes in the character of the responding muscle reactions to stimulation. However, an attempt to analyze this problem, using the same subjects, in the presence or absence of a connection between these two parameters (structure and function), is essential for resolving the question on the development of aging.

EXPERIMENTAL METHOD

A complex study of neuromuscular preparations was carried out on 39 white rats. There were 5 animals between 2 and 6 months of age, 2 between 1½ and 2 years old, and 32 that were 2-3 years or older. After determining a series of physiological indices, the absolute and relative refractivity, conduction rate in the nerve, stimulation thresholds, Vvedenskii's pessimum, and membrane potential [3, 4, 5], the rat was sacrificed (ether), the gastrocnemius and tender muscles were removed and subjected to histological treatment and study.

The histological preparations were impregnated with silver nitrate by the method of Gros-Shul'ttsa, a portion of them were stained with hematoxylin-eosin, and also by the method of Van Guisen.

EXPERIMENTAL RESULTS

The functional peculiarities of the neuromuscular apparatus in aging animals has been considered in previous

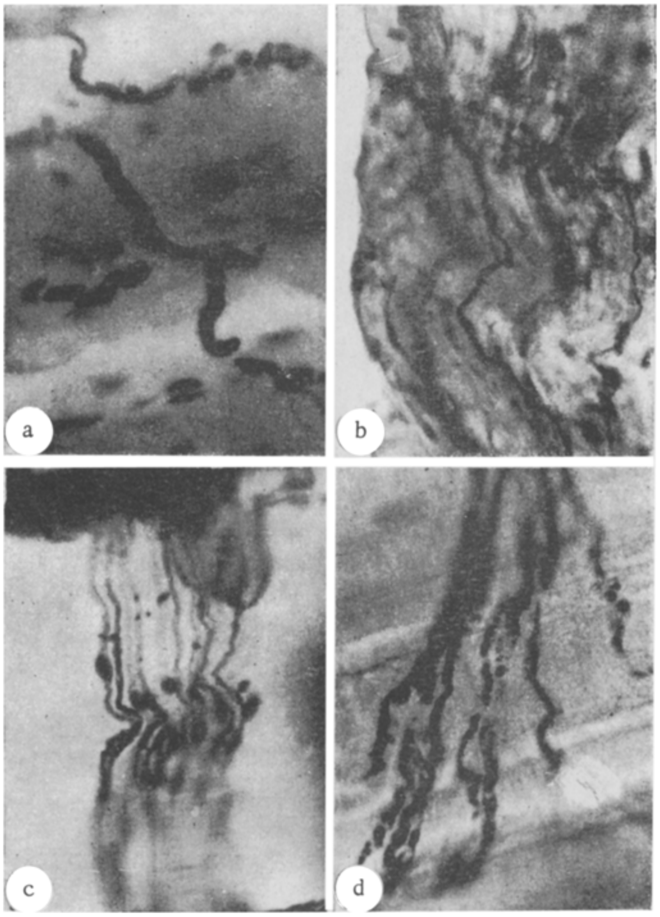

Fig. 1. State of the neural apparatus (morphological picture) in a rat aged $1\frac{1}{2}$ -2 months. a) Uneven thickening and coarsening of the preterminal portions of nerve fibers (Gross-Shul'ttsa. Ocul. 8, obj. 40); b) uneven, primarily weak, impregnation of the fibers in the nerve trunk of larger caliber (Gross-Shul'ttsa. Ocul. 8, obj. 40); c) rather weak impregnation of the nerve fibers in a bundle of medium caliber. In places, the fibers appear as "shadows" (Gross-Shul'ttsa. Ocul. 20, obj. 20); d) clumped disintegration of the nerve fibers (Gross-Shul'ttsa. Ocul. 8, obj. 40).

works [4, 5, 6], and thus, we will not dwell on this question in detail. The changes include a longer absolute and relative refractivity, a decrease in the membrane potential of the muscle fiber, easy formation of Vvedenskii's pessimum, the production of total tetanus with a slower stimulation rhythm than in young animals, elevation of thresholds, and a reduction in oxidative processes.

In the young and sexually mature rats, no peculiarities were noted in the histological preparations, which corresponds to their complete functional capacity.

In the rats aged 2 years and $1\frac{1}{2}$ months it was already possible to detect some signs of physiological and morphological changes. The absolute (2.24 msec) and relative (6.64 msec) refractory phases were prolonged, the stimulation threshold was somewhat elevated; the frequency pessimum, however, developed only with great frequencies – 60-100, which is also characteristic for the young age. In certain muscle fibers with clear cross striation, an increase in the number of nuclei was demonstrated histologically. This is a very important fact, and we consider it one of the early signs of initial aging. The preterminal nerve fibers, showing non-uniform thickness in a number of cases, were tortuous, and coarse thickenings were often observed in them (Fig. 1 a).

In the old rats, a number of peculiarities were noted, both in the muscle fibers and in the preterminal nerve

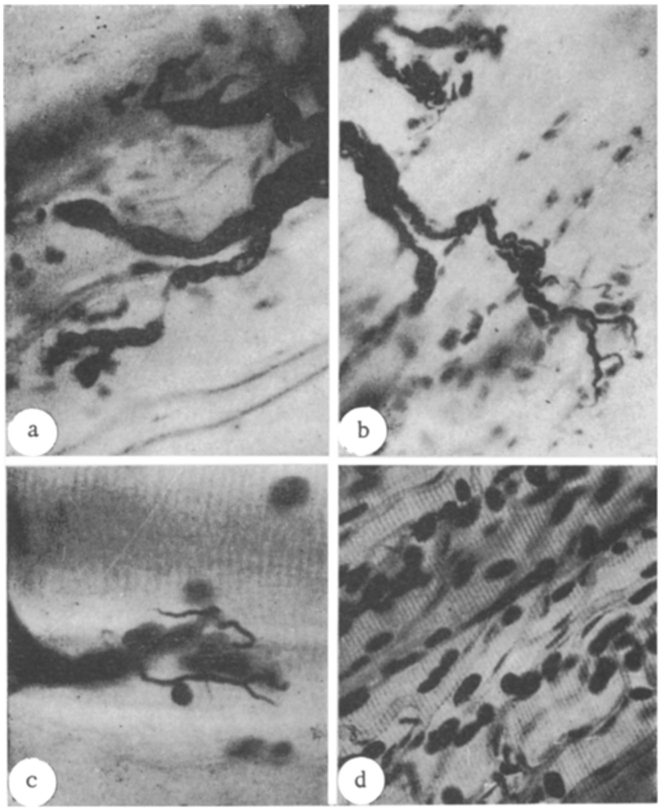

Fig. 2. Morphological picture in the neural apparatus of a rat aged $1\frac{1}{2}$-2 months. a) Uneven thickening and coarsening of the preterminal portions of nerve fibers (Gross-Shul'ttsa. Ocul. 8, obj. 40); b) coarsening and filamentation of the terminal endings of a motor plaque (Gross-Shul'ttsa. Ocul. 20, obj. 20); c) unchanged motor plaque (Gross-Shul'ttsa. Ocul. 20, obj. 40); d) portion of the gastrocnemius muscle with a mass of nuclei. Clear cross striation, obvious unaltered capillaries (Gross-Shul'ttsa. Ocul. 10, obj. 40).

dendrites and nerve endings. In a number of the larger nerve trunks, the nerve fibers were non-uniform in thickness (dilated and narrowed portions), and were either unevenly or weakly impregnated with silver (Fig. 1, b). In certain areas of many mid-caliber nerve bundles the fibers were impregnated so weakly that, in places, only "shadows" could be discerned (Fig. 1 c). In the animals aged 3 years or more, fragmentation of the fibers was noted relatively often. The finer, chiefly preterminal, nerve fibers were markedly deformed, intensely tortuous, and copiously impregnated with silver; large thickenings were often present in them, with uneven contours (this was especially true of the end portions of the preterminal fibers, near the motor plaques).

In a number of the fine nerves, a varying degree of silver impregnation was observed in the course of the same fiber, with clumping disintegration of the membrane and axial cylinder (Fig. 1, d); sometimes we noted vacuolization of fine, pulpy, nerve fibers, with a certain coarsening of the terminal branches in the motor plaques, and in a number of areas – their death (Fig. 2, a). We also encountered roughened motor plaques, with thickening and filamentation of the terminal branches (Fig. 2, b). However, it was always possible to find fine nerve fibers and motor plaques which had undergone absolutely no changes, or almost none at all.

In the sensory nerve fibers that entwine around blood vessels, as well as individual muscle fibers, we usually did not observe marked morphological changes, with the exception of a certain coarsening.

The muscle fibers were thinner; in the fixed preparations, as a rule, their cross-section did not exceed 40 micra. The longitudinal and cross striation was clearly manifested (the latter varied with different fibers). The nuclei of the muscle fibers were usually well stained. In many preparations, wide areas were noted in the muscle fibers with a marked increase in the number of nuclei; at these sites, the cross striation was clearly observed. In certain muscle fibers, the nuclei were markedly elongated, thinned, and arranged in a chain. The muscle fibers were often poorly stained, and contained a small number of nuclei that were round or irregularly-round in shape. The small blood vessels and capillaries entwining the muscle fibers did not undergo morphological changes. Between bundles of muscle fibers we noted an increase in the amount of connective tissue, containing many thin and delicate collagen fibers, and perivascular adipose tissue.

No destructive changes were observed in the sciatic nerve. This completely coincides with the fact, established by us and a number of other authors, that there are no changes in the conduction rate within the nerve trunk [4, 8].

The character of the changes in structure and function are most conveniently considered in individual examples.

Example 1. In a rat aged 3 years, 2 months, we noted a large number of thinned muscle fibers, with an average diameter equal to 66.5 micra (fresh preparation). The membrane potential of the muscle fibers in this muscle were low – 61 microvolts, against a normal of 78-90 microvolts. In this animal, the pessimum developed easily with transition of the stimulation frequency from 40 to 100/sec. Histologically, we found destructive changes in the nerve fibers, the nerve endings, and the motor plaques, manifested to a stronger or weaker degree in different areas.

Example 2. In a female aged 3 years, with clearly demonstrated signs of aging, fatigue set in rapidly upon tetanic stimulation of the nerve. Total tetanus arose with a slow impulse frequency – 30/sec, and the pessimum easily arose with an increase in the frequency of the impulses to 80/sec. In the gastrocnemius muscle, histological investigation showed weak silver impregnation of the fine nerve fibers and motor plaques, and in certain cases – their disintegration. The muscle fibers were atrophic, 40-45 micra in thickness (fixed preparation), and their cross striation was expressed weakly. The profound senile changes present in the neural conductors and motor plaques of this rat explain, to a certain degree, the physiological features of the muscle's response to frequent stimulation.

Example 3. In a female aged 3 years, 2 months, total tetanus arose with 40 impulses/sec. A weak pessimum developed only at 100 impulses/sec, but was clearly manifested at 150/sec. The mean diameter of the muscle fiber in the fresh preparation was 55 micra. In the histological preparations of this animal, along with nerve fibers that were weakly impregnated with silver, there were also well stained fibers with thickenings in the preterminals. Many motor plaques remained unchanged (Fig. 2, c). The muscle fibers were characterized by clear cross striation, and there was a marked increase in the number of nuclei (Fig. 2, d). In this case, despite the age of the rat, the morphological and physiological indices were close to those in the younger rats. It is especially important to emphasize the absence of changes in many motor plaques, which coincides with the development of the pessimum only at high frequencies, characteristic of young animals.

It is obvious from the examples presented that destructive changes in the motor plaques play an essential role in the development of the pessimum. The more profound and more clearly manifest their dystrophy, the easier the pessimum developed with slow stimulation frequencies.

It may be postulated that profound changes in the condition of the muscle arise specifically in those fibers whose innervation is disrupted, which is observed from studying the histological preparations. It is known [10, 12] that, with aging, the number of nerve cells in the spinal cord decreases, and the number of nerve fibers in the anterior and posterior radicles is reduced by 10% [11]. On the basis of this, it may be postulated that destructive changes in the neural elements of the muscle are a result and a reflection of changes occurring in the motor neuron. But with this hypothesis, logically following from our knowledge on this question, it still remains unclear why changes are not observed in the axons that make up the nerve trunk and innervate the given muscle, and why the destructive changes occur, in the first order, in the preterminal nerve fibers and nerve endings. This can apparently be explained by the fact that the axons of individual motor neurons (which die soon after the start of the investigation), located in the common trunk of the nerve, remain unnoticed in its total mass, which contains up to 6000 fibers [9]. On the periphery of the muscle, where the axon branches into finer nerve twigs, the destructive changes are clearly seen. Granular and clumped disintegration of the nerve elements, which was observed in a number of the preparations from the aging rats, testified to the fact that these elements had already died.

Analogous or close changes are noted in the neurla conductors and endings with certain pathological conditions, including tuberculosis, where they are explained by hypoxia [2, 7]. It is possible that some morphological changes that we observed are also related to tissue hypoxia. Decreased utilization of oxygen by the tissue was encountered in the muscles of old rats, which is explained by a weakened course of certain enzymatic processes.

SUMMARY

The authors compared some physiological indices of the neuromuscular system of old animals with histomorphological peculiarities of muscles and their neural structures. 2-3 year-old albino rats were used as control animals. Structural alterations in neural elements and muscle fibers were observed in old animals. Considerable changes were noted also in preterminal nerve fibers and motor plaques (coarsening, deformities, weak and irregular impregnability with silver, destruction). There was also observed an augmented number altered form and irregular staining of nuclei in muscle fibers. The rate of physiological changes during aging (low membrane potential, high values of absolute and relative refractory phases, easy formation of Vvedenskii's pessimum) is directly related to the intensity of structure alterations. It was found that the destruction of nervous elements appears earlier than that of muscle fibers.

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