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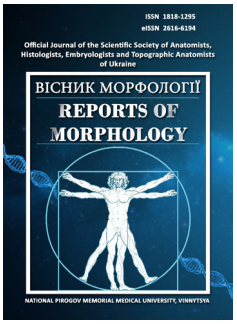

Morphological changes in the extraorgan vascular bed of the pineal gland of rats under conditions of chronic stress

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DATA SHARING
Data are available upon reasonable request to corresponding author.

Modern Ukrainian society has been in conditions of active military operations for several years, which have caused disruption of sleep patterns, lack of proper rest, and a sense of security, which has led to a state of chronic stress. The main role in protecting the body from stress factors, regulating sleep, and implementing adaptive reactions belongs to the pineal gland and the hormone it synthesizes – melatonin. However, prolonged exposure to stress factors leads to disruption of the functional activity of the pineal gland, which manifests itself not only at the cellular level but also in the state of the vascular bed. The aim of the work was to study morphological changes in the state of the extraorgan vascular bed and rheological properties of the pineal gland blood under conditions of chronic stress. The study was conducted on 12 adult white male Wistar line rats, which were divided into control and experimental groups. Animals in the control group were kept under normal vivarium conditions without the influence of additional factors. Animals in the experimental group were subjected to chronic stress by forced swimming for 60 minutes a day for 10 days. To study the state of the vascular bed of the pineal gland, morphological, morphometric, and statistical research methods were used. During the study, morphological manifestations of extraorgan blood circulation disorders in the pineal gland were detected, manifested by changes in the rheological properties of the blood and the restructuring of the walls of venous and arterial blood vessels. It was established that violations of the rheological properties of blood in venous vessels were manifested by blood separation, aggregation and lysis of erythrocytes and stasis, and in arterial vessels – by the practically absence of erythrocytes in the lumen of the vessels. Morphological changes in the state of the vascular wall in the veins were manifested by endothelial cell hypertrophy, stretching and thinning of the vascular wall, and its ruptures. In arterial type vessels, hypertrophy and edema of endothelial cells and spasm of muscle cell membranes were detected. Thus, the detected morphological changes indicate impaired blood circulation and slowing of blood flow, which leads to hypoxia of the pineal gland parenchyma.

Keywords: *chronic stress, pineal gland, blood vessels, rheological properties of blood.*

Introduction

The conditions of the modern reality of Ukrainian society, which has been in conditions of active military operations for several years, are characterized by socio-economic difficulties and uncertainty of the situation, which undoubtedly affects the state of mental health of the population [9, 14]. In addition, all these factors, against the background of the sounds of explosions, sirens, sleep disturbances, lack of proper rest and a sense of security, cause a state of constant stress in all segments of the population [20, 22, 23]. It is known that chronic stress, in its various manifestations, remodels brain homeostasis, which negatively affects the psychological state, is accompanied by depressive disorders, cerebral circulation disorders, premature brain aging and atrophy, which ultimately leads to functional and morphological restructuring of all body systems, the development of various diseases, impaired cognitive functions and a decrease in the quality of life [26, 29]. The main role in protecting the body from stress factors, regulating sleep, and implementing

adaptive reactions belongs to the organ of the central neuroendocrine system – the pineal gland and the hormone it synthesizes – melatonin [6, 8]. It is known that melatonin has a wide spectrum of biological activity, is a universal adaptogen that has a protective effect on all organs under stress and slows down the development of pathological changes in the body [18, 19, 30]. Therefore, prolonged exposure to stress factors leads to pineal dysfunction, which is accompanied by a decrease in melatonin secretion, sleep disorders and neurological disorders [2, 7, 24].

In recent years, an increasing number of scientific publications have been devoted to the study of changes in the morpho-functional features of the pineal gland under the influence of pathological factors, including stress [21]. At the same time, there are few among them that consider and investigate the morphological manifestations of changes in the vascular bed and rheological properties of the blood of the pineal gland, which arise against the background of chronic stress and can lead to inhibition of the functional activity of the organ and psychoneurological disorders [12, 13].

Therefore, *the aim* of our study was to study morphological changes in the vascular bed and rheological properties of pineal gland blood under conditions of chronic stress.

Materials and methods

The results of this work are a fragment of the research topic of the Department of Morphology and Public Health of the Petro Mohyla Black Sea National University of the Ministry of Education and Science of Ukraine “The influence of environmentally hazardous factors on the mechanisms of development of civilization diseases and their correction with physiologically active substances”, state registration number 0124U002163.

All stages of the study, manipulative interventions and euthanasia of animals were carried out in compliance with the requirements and general principles of work with experimental animals in accordance with the following standards: Council of Europe Convention on Bioethics (1997); European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, General Ethical Principles of Experiments on Animals, approved by the First National Congress of Ukraine on Bioethics (2001); Law of Ukraine “On the Protection of Animals from Cruelty” (2006) and other international treaties and current national legislation in the field of biomedical research. The study was conducted according to a protocol approved by the Bioethics Commission of the Petro Mohyla Black Sea National University (Protocol No. 4 dated June 24, 2024).

The experimental study involved 12 sexually mature laboratory rats of the Wistar line, weighing 200-220 g. The choice of males for the study was due to the absence of cyclic fluctuations in blood plasma melatonin levels compared to females, in which the content of this hormone, and therefore the morphological and functional state of the pineal gland, is determined by the phase of the ovarian cycle [16, 27].

The experimental animals were kept in standard vivarium conditions throughout the study and had free access to food and clean water. Artificial lighting sources were not used in the vivarium, since the pineal gland, which is the main source of endogenous melatonin, reacts to changes in light levels with morpho-functional restructuring, which is accompanied by fluctuations in the level of the hormone in the blood plasma [11, 15]. Therefore, the research was performed under natural lighting typical of the autumn-winter period.

To study the effect of chronic stress on the state of the vascular bed of the pineal gland, two groups of animals were formed: control and experimental. Each group included six rats, which is the minimum acceptable norm for the number of animals required for statistical research. The animals in the control group were kept in standard vivarium conditions and were not exposed to additional factors, since any, even minor, changes in maintenance or manipulation are a stressful factor for the animals and can cause morphological changes in the pineal gland, which is responsible for the start of the stress response and adaptation processes. The animals in the experimental group were simulated to experience chronic stress through forced swimming. For this purpose, the rats were placed in a tank with 10 liters of water for 1 hour. The water temperature was maintained within 28-30 °C, and the room temperature was 25 °C [3]. One-time training sessions were carried out for 10 days.

Rats from the control and experimental groups were removed from the experiment simultaneously, on the 1st day after the last forced swim, by performing a single-stage decapitation under thiopental anesthesia, which was administered intraperitoneally at a rate of 25 mg/kg of animal weight. After the decapitation procedure was completed, the animals’ skulls were scalped with subsequent removal of its vault along with the dura mater. Then the brain was separated together with the pia mater from the base of the skull and fixed in 10 % neutral formalin solution for 20 hours. After washing the fixed material and isolating the pineal gland, dehydration was carried out in alcohols of increasing concentration. Using standard methods, the obtained material was embedded in paraffin blocks, from which sections 5-6 μm thick were made on a semi-automatic rotary microtome “Microm” (Germany) and stained with hematoxylin and eosin in accordance with generally accepted methods. The obtained histological preparations of the pineal gland were studied and photographed at a magnification of ×10 binoculars, ×10, ×20, ×40 objective lenses of a microscope of the “Carl Zeiss” brand (Germany). Photo documentation of the research results was performed using a Canon digital camera.

To assess the morpho-functional state of the blood vessel wall of the pineal gland, the area of the endothelial cell nuclei was determined by measuring their large and small diameters. Morphometric measurements were performed using a screw-type ocular micrometer MOV 1-16 at an objective magnification of ×40.

Statistical calculations were performed on a personal computer using the “STATISTICA 10” software for computers

with the Windows operating system. The numerical data obtained during morphometric measurements were processed using standard statistical methods, calculating the arithmetic mean, standard error of the arithmetic mean, and standard square deviation. The results are presented as M±m. Student's t-test was used to assess the significance of differences between groups. The difference was considered significant when the numerical parameters between the control and experimental groups differed at a level of value not less than $p<0.05$.

Results

Histological studies of pineal gland preparations showed that in the control group of animals, extra-organ blood vessels had an intact appearance and uniform blood distribution. It was found that the walls of blood vessels were characterized by a typical three-layer structure and consisted of intima, media, and adventitia. The intima was represented by evenly spaced endothelial cells. The nuclei of endothelial cells were characterized by predominantly round shape. According to the results of morphometric measurements, it was found that the average area of the nuclei of endothelial cells of large-caliber venous vessels was 12.69±0.43 μm^2, and of arterial vessels – 18.06±0.52 μm^2. The middle layer of blood vessels consisted of layers of smooth muscle cells, the number of which depended on the caliber and type of vessel. As the diameter of the arteries increased, the number of muscle cell layers increased accordingly. The middle layer of venous vessels, in addition to smooth muscle cells, contained elastic fibers. The adventitia was formed by loose connective tissue. It should be noted that the walls of blood vessels of both arterial and venous types were not thickened and without ruptures (Fig. 1).

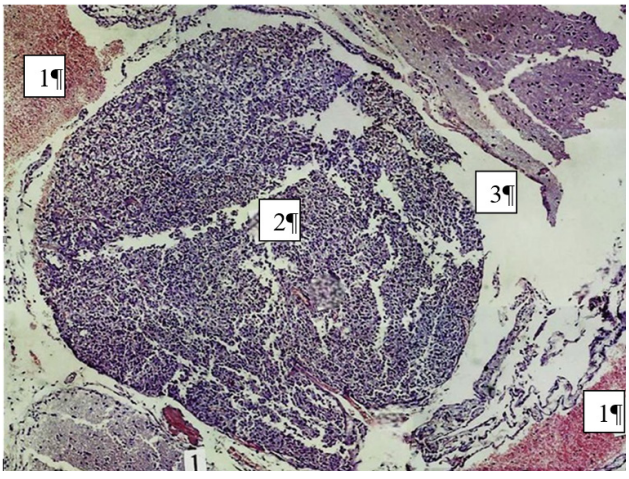

Fig. 1. Micrograph of the pineal gland of laboratory rats of the control group: 1 –extraorgan blood vessels; 2 – pineal gland; 3 – subarachnoid space. Staining: hematoxylin and eosin, ×100.

During microscopic examination of the preparations of rats of the experimental group, it was found that daily forced swimming for 10 days causes pathological changes in the blood vessels of the pineal gland, as an organ that plays a major role in ensuring the adaptation of the organism in response to the influence of stress factors, which leads to its morphological restructuring and changes in functional activity. Thus, during the light-optical study of extra-organ blood vessels, we detected morphological manifestations of changes in the structure of their walls and disturbances in the morphological manifestations of the rheological properties of blood.

In the lumen of large-diameter extra-organ veins, a fairly clear separation of blood into plasma and erythrocyte mass is noted. Moreover, it should be noted that the plasma component of the blood acquired a pronounced eosinophilic color and a homogeneous appearance, which may indicate coagulation of blood plasma proteins. Upon further examination of the erythrocyte mass, it was found that it was a conglomerate formed by single isolated erythrocytes with varying degrees of staining. Some erythrocytes were bright red, while others were pale pink. In some places in the studied conglomerate, clumps of adhered erythrocytes were found, the gaps between which were practically indistinguishable. In such vessels, adhered erythrocytes blocked the lumen of the venous vessel, which was accompanied by blood stasis. When examining extra-organ vessels of the venous type of small diameter, manifestations of erythrocyte aggregation were also detected. The wall of such blood vessels and endothelial cells underwent changes. Endothelial cells were arranged chaotically, which led to an increase in the distance between neighboring endothelial cells, thinning of the vascular wall, and the appearance of defects and ruptures, as a result of which blood plasma and single erythrocytes entered the extravascular space (Fig. 2). Ruptures of the vascular wall were also found in large diameter veins.

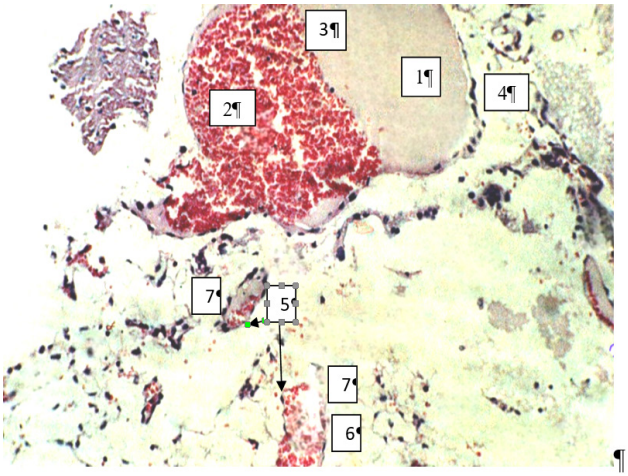

Fig. 2. Micrograph of extraorgan blood vessels of the pineal gland of venous type during chronic stress in rats: 1 – large diameter vein; 2 – erythrocyte mass; 3 – blood separation; 4 – vein wall; 5 – small diameter veins; 6 – nature of the distribution of blood elements in small veins; 7 – rupture of the venous vascular wall. Staining: hematoxylin and eosin, ×200.

In addition, in venous vessels, morphological manifestations of impaired rheological properties of blood

were detected, which were manifested by the phenomena of erythrocyte lysis, which appeared on histological preparations in the form of a pale pink homogeneous mass. It was established that such an erythrocyte mass occupied either the central zone of the blood vessel lumen or was closely adjacent to the vessel wall, which was in contact with the capsule of the pineal gland. It was found that the blood plasma in such vessels appeared transparent, indicating the absence of protein coagulation. It was found that the lumens of blood vessels in certain places had expansions of different sizes, formed exclusively in the wall that was not adjacent to the organ capsule. In such areas of the venous wall, its thickening and the reaction of the endothelial layer are noted, in the form of convergence of adjacent endothelial cells and an increase in the size of their nuclei (Fig. 3). Further histological examination revealed that the nuclei of endothelial cells of venous vessels acquired an oval shape, and their average area was 15.96±0.61 μm^2, which exceeded the control values by 25.77 % ($p<0.05$) and indicated edema and hypertrophy.

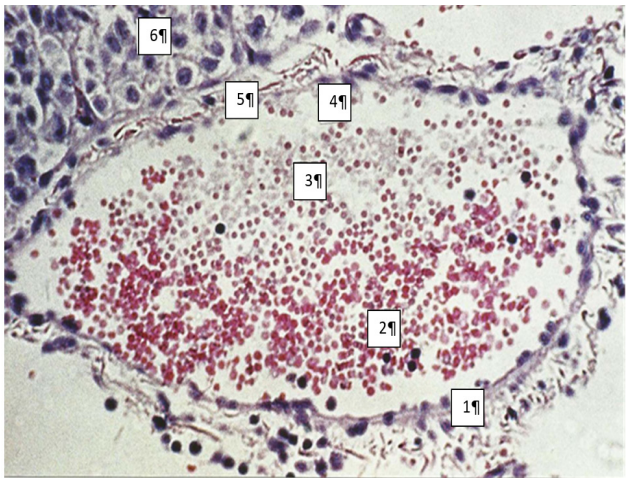

Fig. 3. Micrograph of a longitudinal section of a venous-type vessel adjacent to the pineal gland capsule of a rat under chronic stress: 1 – wall of an extraorgan vein; 2 – intact erythrocytes; 3 – lysis of erythrocytes; 4 – convergence of adjacent endothelial cells; 5 – pineal gland capsule; 6 – pineal gland parenchyma. Staining: hematoxylin and eosin, ×400.

When studying extra-organ vessels of the pineal gland of arterial type of different diameters, we also found signs of hemocirculatory disorders. It was found that the arterial vessels appeared ischemic. There were practically no erythrocytes in their lumens. Only single arterioles were filled with erythrocytes. It was found that the hemorrhage of arterial vessels on longitudinal sections was expressed over a significant area (Fig. 4).

It is also necessary to note morphological changes in the structures of the vascular wall of arterioles. The reaction of endothelial cells was primarily observed. They had an elongated shape, appeared hypertrophied with sharply enlarged dark nuclei filled with heterochromatin. It was found that the area of endothelial cell nuclei increased by 30.73 %

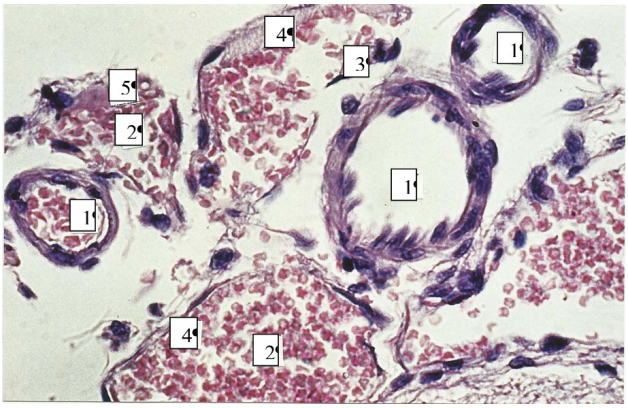

Fig. 4. Micrograph of extraorgan blood vessels of the pineal gland of rats under chronic stress: 1 – arteriole lumens; 2 – vein lumen; 3 – ruptures of the vein wall; 4 – erythrocyte adhesion; 5 – erythrocyte lysis in the parietal zone. Staining: hematoxylin and eosin, ×400.

($p<0.05$) compared to the control values and amounted to 23.61±0.82 μm^2. It was noted that the nuclei of endothelial cells protruded into the lumen of the blood vessel. Along with this, it is necessary to note changes in the muscular layer of the blood vessel wall. The smooth muscle elements of the media were in a state of spasm, which was accompanied by a decrease in the lumen of the vessel.

Discussion

The presented study was aimed at supplementing data on pathological changes in the vascular bed of the pineal gland and the rheological properties of blood in laboratory Wistar rats under conditions of chronic stress, since information on the state of the vascular bed in the domestic and foreign literary sources we have reviewed is sparse, which is due to both the small size of the gland and the complex topographic features of its location in the brain, which complicates atraumatic access to it [5, 10, 17]. In addition, there is no data in the literature on the state of endothelial cells of the pineal gland vessels, which play an important role in maintaining vascular wall tone and ensuring adaptation to hemodynamic changes [25, 28].

The morphological studies conducted showed that chronic stress simulated by forced swimming was accompanied in experimental animals by pronounced disorders of extra-organ blood circulation in the pineal gland, which were manifested both at the level of venous and arterial links. Thus, in the extra-organ venous bed we detected pathomorphological changes in the rheological properties of blood, manifested by blood separation, aggregation and lysis of erythrocytes and stasis, which indicates impaired blood circulation and slowing of blood flow [4, 12]. Disturbances in the rheological properties of blood led to morphological changes in the structure of the venous vessel wall, manifested by hypertrophy of endothelial cells, an increase in the distance between adjacent cells, stretching and thinning of the vascular wall and its ruptures, which contributed to the development of plasmorrhagia and

edema [12, 13].

Manifestations of chronic stress were also detected in extra-organ vessels of the arterial type and were manifested by the almost complete absence of erythrocytes in the lumens of the vessels, which indicates impaired oxygen transport, the development of hypoxia of pinealocytes and a decrease in the functional activity of the organ [12, 13]. Given the anatomical features of the pineal gland's location in the brain and its close connection with it, insufficient oxygen supply to the gland can lead to hypoxia, brain damage, and decreased brain function [1]. The impact of chronic stress was also accompanied by pathomorphological changes in the arterial vessel wall. It was found that the nuclei of endothelial cells were edematous, and as a result increased in size relative to the control group by 30.73 %. The nuclei of endothelial cells were dark because they were filled with functionally inactive heterochromatin, which stains well with basic dyes. Morphological changes in the muscular layer of the arterial wall, under conditions of chronic exposure to stress factors, were manifested by spasm and a decrease in the lumen of the corresponding vessel, which leads to impaired hemocirculation and ischemia.

Thus, the results obtained by us coincide with the results of scientific works of other researchers who studied the influence of chronic stress of various genesis on the state of the pineal gland and indicate that the prolonged exposure to stress factors is accompanied by pathological changes in the morphology of the wall of the blood vessels of the pineal gland, impaired rheological properties of blood and blood circulation, which leads to hypoxia of the organ parenchyma, changes in the structural and functional properties of pineal cells, their apoptosis, a decrease in the activity of the organ and disruption of adaptive processes [12, 13].

This experimental article differs from those published in this field in that it is the first to describe and analyze changes in the vascular bed of the pineal gland of laboratory Wistar rats under conditions of chronic stress caused by excessive physical exertion. The morphological analysis performed may be one of the criteria for assessing the functional state of the pineal gland under the influence of pathological factors in animals, which are most often used in experimental studies.

Conclusions

1. Chronic exposure to stress factors leads to pathomorphological changes in the condition of the walls of extra-organ blood vessels. In venous vessels, changes in the endothelium were detected, which were manifested by hypertrophy of endothelial cells, an increase in the distance between adjacent cells, stretching and thinning of the vascular wall and its ruptures, which contributed to the development of plasmorrhagia and edemaIn arterial type vessels, hypertrophy and edema of endothelial cells and spasm of muscle cells were detected, which was accompanied by a decrease in the lumen of the arterial vessel, which can lead to impaired blood outflow and ischemia of the pineal gland tissue.

2. Morphological changes in the rheological properties of blood in extra-organ venous vessels have been identified in the form of blood separation, aggregation and lysis of erythrocytes, and stasis, which indicates impaired blood circulation and slowing of blood flow.

3. In extra-organ vessels of the arterial type, manifestations of chronic stress were detected in the form of the practically absence of erythrocytes in the lumens, which indicates a violation of oxygen transport, the development of hypoxia of pinealocytes and a violation of the functional activity of the pineal gland.

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МОРФОЛОГІЧНІ ЗМІНИ ЕКСТРАОРГАННОГО СУДИННОГО РУСЛА ШИШКОПОДІБНОЇ ЗАЛОЗИ ЩУРІВ ЗА УМОВ ХРОНІЧНОГО СТРЕСУ

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Сучасне українське суспільство вже декілька років перебуває в умовах активних військових дій, які стали причиною порушення режиму сну, відсутності повноцінного відпочинку та почуття безпеки, що спричинило стан хронічного стресу. Головна роль у захисті організму від стресових факторів, регуляції сну та здійсненні адаптаційних реакцій належить шишкоподібній залозі та гормону, який вона синтезує – мелатоніну. Однак, тривалий вплив стресових факторів призводить до порушення функціональної активності шишкоподібної залози, що проявляється не тільки на клітинному рівні, а і на стані судинного русла. Метою роботи було вивчення морфологічних змін стану екстраорганного судинного русла та реологічних властивостей крові шишкоподібної залози за умов хронічного стресу. Дослідження проводилося на 12 дорослих білих щурах-самцях лінії Вістар, які були розподілені на контрольну і дослідну групи. Тварини контрольної групи перебували за звичайних умов віварію без впливу додаткових факторів. Тваринам дослідної групи моделювали хронічний стрес шляхом примусового плавання по 60 хвилин на добу впродовж 10 діб. Для дослідження стану судинного русла шишкоподібної залози використовували морфологічні, морфометричні та статистичні методи

дослідження. В ході проведення дослідження були виявлені морфологічні прояви порушення екстраорганного кровообігу у шишкоподібній залозі, що виявлялися змінами реологічних властивостей крові та перебудовою стінок кровоносних судин венозного і артеріального типу. Встановлено, що порушення реологічних властивостей крові у судинах венозного типу проявлялися сепарацією крові, агрегацією і лізисом еритроцитів та стазом, а у судинах артеріального типу – практичною відсутністю еритроцитів у просвітах судин. Морфологічні зміни стану судинної стінки у венах проявлялися гіпертрофією ендотеліоцитів, розтягуванням і потоншенням судинної стінки та її розривами. У судинах артеріального типу виявлена гіпертрофія і набряк клітин ендотелію та спазм клітин м'язової оболонки. Таким чином, виявлені морфологічні зміни свідчать про порушення циркуляції крові та уповільнення кровотоку, що призводить до гіпоксії паренхіми шишкоподібної залози.

Ключові слова: *хронічний стрес, шишкоподібна залоза, кровоносні судини, реологічні властивості крові.*

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Pshychenko V. V. – data visualization, methodology and original project writing.
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