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EXPERIMENTAL EVALUATION OF LASER IRRADIATION EFFECTS ON THE DERMAL MICROCIRCULATORY SYSTEM

ЕКСПЕРИМЕНТАЛЬНЕ ВИЗНАЧЕННЯ ВПЛИВУ ЛАЗЕРНОГО ОПРОМІНЕННЯ НА МІКРОЦИРКУЛЯТОРНЕ РУСЛО ДЕРМИ

Резюме. Висока ефективність та безпечність лазерної терапії забезпечують можливість починати лікування на ранніх стадіях захворювань у пацієнтів різних вікових груп, зокрема у педіатричній практиці. Такий підхід дає змогу впливати не лише безпосередньо на патологічний осередок, а й надійно запобігати потенційним ускладненням, що підкреслює важливість досліджуваної проблеми. Притаманні лазерному випромінюванню переваги щодо лікування судинних утворень шкіри вимагають подальшого детального вивчення з метою визначення оптимальних умов його застосування – вибору типу лазера, раціональних довжин хвиль, потужності та інших біотехнічних характеристик опромінення.
Мета. Дослідити патологічні зміни судин мікроциркуляторної ланки дерми після одноразового впливу лазерного опромінення зі стандартними параметрами потужності та тривалості, а також визначити їхній зв'язок з інволютивними процесами пурпури шкіри.
Матеріал та методи. Проведено аналіз змін гістологічної будови та характеру тканинних реакцій шкіри після послідовного лазерного опромінення через 30 хвилин (ділянка № 1), 60 хвилин (ділянка № 2) і 90 хвилин (ділянка № 3). Експеримент виконано на морських свинках масою 350,0-400,0 г і віком 6-8 тижнів, вибір яких обґрунтований морфологічною подібністю будови їхньої шкіри до людської. Аналіз динаміки морфометричних параметрів мікроциркуляторної ланки дерми показав певну лінійну залежність між ними та величиною відносної площі стромального набряку – одного з ключових компонентів пурпури шкіри – у всі досліджувані часові проміжки. При цьому площа стромального набряку дерми мала тенденцію до збільшення, незважаючи на зменшення видимих проявів пурпури. Виявлена зворотна залежність між клінічною динамікою поверхневих проявів пурпури та вираженістю морфологічних змін у глибших шарах дерми. Через 60 хвилин після опромінення спостерігалися значніше повнокрів'я, периваскулярний набряк і стаз еритроцитів у судинах мікроциркуляторного русла порівняно з 30-хвилинним інтервалом, на тлі помірних пердіапедезних крововиливів, реактивної запальної інфільтрації та пошкодження структурних компонентів сполучної тканини.
Висновки. Поява пурпури шкіри після одноразового лазерного опромінення може розглядатися як клінічно значущий маркер адекватності вибраних енергетичних параметрів впливу. Порівняння динаміки інволюції пурпури з морфологічними та морфометричними показниками судинних змін у всіх шарах дерми свідчить про швидке поширення патологічних процесів на тлі зростання площі стромального набряку вже на ранніх етапах після опромінення. Отримані результати підтверджують можливість формування циклічних патологічних змін структурних компонентів дерми, насамперед мікроциркуляторної та судинної ланок, після кожного наступного сеансу лазеротерапії в межах індивідуальної програми лікування судинних утворень шкіри.
Ключові слова: дерма, судинні утворення, лазери, лікування, експеримент, морфологія шкіри, патологічні зміни, мікроциркуляторне русло.

Modern arsenal of methods for treating vascular lesions of the skinincludes a wide range of approaches – from surgical removal, coagulation, cryodestruction, radiotherapy and chemotherapy to combined methods. The choice of the optimal treatment method is determined by the individual characteristics of the formation: its type, size, localization, patient's age and general health [1].

The most radical method is the complete removal of the pathologically altered area with partial involvement of adjacent healthy tissues through a surgical incision. However, this approach is not always advisable, especially when the lesions are located in cosmetically sensitive or functionally important areas, as it can lead to structural defects and aesthetic losses, which negatively affects the patient's quality of life and psychological comfort.

In order to minimize such risks, the search for low-traumatic therapy methods continues, among which modern researchers recognize the use of laser radiation as one of the most promising.

Laser therapyis considered the «gold standard» for the treatment of superficial vascular neoplasms of the skin. According to the theory of selective photothermolysis (SPT), which is based on the selective absorption of light by biological structures, laser irradiation causes irreversible thermal damage to abnormal vessels with minimal impact on the surrounding skin. The pronounced absorption of the laser pulse by hemoglobin can lead to the evaporation of water from the blood and the formation of local hemorrhage, which manifests itself in the form of purpura, which confirms the connection of this phenomenon with the mechanisms of SPT [2, 3].

Current experimental data suggest that the skin remains perfused after laser exposure even in the absence of visible signs of purpura (the characteristic dark shade persists for about a month after the procedure). Therefore, the presence of purpura is usually considered as the final clinical criterion for the adequacy of treatment and as a positive prognostic indicator [3, 4].

Taking into account the assumption that the main layers of the skin – the epidermis and dermis – can be considered as solid homogeneous structures with low water content, thermal damage to these layers can be used to assess the safety of laser exposure. Based on this concept, it is believed that the area of purpura corresponds to the area of hemorrhage according to the dorsal skin chamber (DSC) model, which is effective for studying the thermal response of vessels, and is directly proportional to the volume fraction of the tumor vascular network in the laser exposure zone [4, 5].

Benefits of laser therapyregarding its effectiveness and safety allow it to be used in the early stages of diseases in patients of all ages, including children. Laser exposure not only acts directly on the pathological focus, but also prevents the development of possible complications, which confirms the relevance of further research in this area. However, the potential of laser radiation in the treatment of vascular lesions requires deeper scientific justification – in particular, clarification of the type of laser, rational wavelengths, power parameters and other biotechnical characteristics to achieve maximum therapeutic effect.

The aim of the study is to investigate pathological changes in dermal microcirculatory vessels after single exposure to laser irradiation with standard power and duration parameters, and to determine their relationship with involutive processes of skin purpura.

Material and methods. Changes in the histological structure and the nature of tissue reactions of the skin after laser irradiation were studied after 30 minutes (area № 1), 60 minutes (area № 2) and 90 minutes (area № 3). Statistical analysis was performed in groups of patients comparable in quantitative and qualitative characteristics. The distribution of participants into groups was carried out by the «blind» method, which ensured the minimization of the influence of stratifying factors. Data processing was performed using the Statistica 6.0 for Windows software packages and the licensed version of BioStat.

The experimental part was conducted on guinea pigs weighing 350.0-400.0 g and aged 6-8 weeks, whose selection was due to the morphological similarity of their skin to human skin.

The study was conducted in accordance with the European Convention for the Protection of Vertebrate Animals in compliance with the basic requirements of GLP (1981). The Bioethics Commission of the National Pirogov Memorail Medical University confirmed the study's compliance with ethical and legal norms in accordance with the order of the Ministry of Health of Ukraine No. 281 dated 01.11.2000. All animals were kept in vivarium conditions during the two-week quarantine and throughout the experiment, received a standard diet, and water was provided without restrictions. Their health and age were assessed by a veterinary specialist.

The distribution of animals according to the chosen research design according to the stages of the experiment is presented in Table 1.

Two intact animals were selected as controls, from which skin samples were taken from the back, similarly to the rest of the animals.

Conceptual views on the irradiation of vascular tumors of the skin are in the plane of the feasibility of using lasers, since their energy is maximally absorbed by the melanin of the epidermis, which was convincingly proven by the studies of N. Jia et al., (2019) (Table 2) [6].

Table 1

Experimental study design

Research terms	Stages of experimental research
30 minutes after irradiation	Stage I of the experiment (n=5)
	15 biopsies
60 minutes after irradiation	Stage II of the experiment (n=5)
	15 biopsies
90 minutes after irradiation	Stage III of the experiment (n=5)
	15 biopsies
In general	45 biopsy specimens

Table 2

Biotechnical parameters during laser irradiation of the skin

	Indicator	Derma	Blood
Optical properties	Absorption coefficient, (μA/cm^{-1})	20	49.3
	Scattering coefficient, (μs/cm^{-1})	460	466
	Anisotropy index, (g)	0.8	0.995
	Refractive index, (n)	1.37	1.33
Thermal properties	Density, (g/kg×m^{-3})	1090	1060
	Thermal conductivity, (k/kW×m^{-1}×K^{-1}	0.41	0.55
	Specific heat capacity, (in/J×kg^{-1}×K^{-1})	3500	3600

In order to unify the conditions of energy impact on each of the skin areas of experimental animals, a fixing device of our own design was used. This device is a special fixing system that includes a tripod object platform (1), on which the laboratory animal is held by all four limbs (including in a state of anesthesia) with the help of clamps. On the platform is a previously prepared area of skin (2), cleaned of hair and treated with an antiseptic solution to prevent bacterial contamination.

A horizontally oriented flexible mesh plate with a standard cell area of 1 cm² (4) is attached to the support axis of the tripod (5) using a fixing unit (6). Due to the ability to change its profile in accordance with the anatomical configuration of the animal's body, additional stabilization of the study area on the platform surface is provided.

An additional tripod (7) with a fixing screw (8) is fixedly fixed in the upper part of the object platform, which holds the horizontally oriented end of the emitter (3). The emitter is connected to the laser device by a flexible fiber optic cable (9), which allows precise adjustment of the position of the emitter end relative to the skin surface and ensures a standardized nature of the laser exposure (Fig. 1).

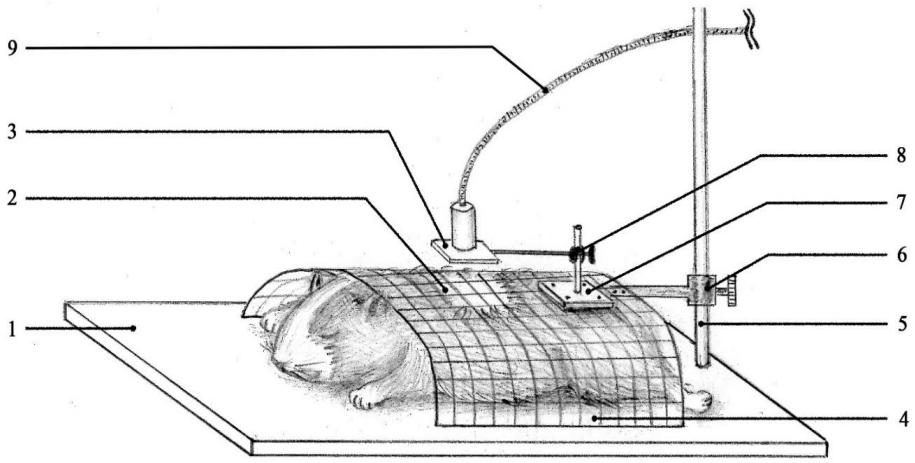


Fig. 1. Scheme of the device for standardizing the amount of energy injury to the skin by laser irradiation in an experiment on laboratory animals: 1 – object platform of the tripod; 2 – prepared area of skin; 3 – end of the emitter; 4 – flexible mesh plate; 5 – support axis of the tripod; 6 – fixing unit; 7 – additional tripod; 8 – fixing screw; 9 – flexible fiber optic cable

The laboratory animal was fixed by four limbs in a prone position on the object platform of the tripod (1). Additional stabilization was provided from above by a flexible mesh plate with a standard cell area of 1cm² (4), which was previously modeled according to the contours of the body. The position of

the plate was maintained by means of a fixing unit (6) installed on the support axis of the tripod (5).

A horizontally oriented end of the emitter (3) was placed above a prepared area of skin (shaved and treated with an antiseptic to prevent bacterial contamination) in the back area (2). Its fixation was provided by an additional tripod (7) with a fixing screw (8). A flexible fiber optic cable (9) was connected to the end of the emitter, which performed switching with the laser device and minimized the scattering of laser radiation outside the clearly defined experimental irradiation zone.

The proposed method received a Certificate of Ukraine on registration of copyright for the work № 133258 «Method of standardizing the magnitude of energy injury to the skin by laser irradiation in an experiment on laboratory animals» dated February 6, 2025.

Immediately 60 minutes before the irradiation session, EMLA cream (Sweden) was applied around each selected area of skin, outside the marked circle, for the purpose of local anesthesia (Fig. 2).

Energy irradiation of the skin was performed using a pulsed laser Candela™ Vbeam Perfecta (USA) with a wavelength of 595 nm, a pulse duration of 0.45 ms, and a power of 9.5 J. The duration of one irradiation session in all cases was 15 minutes, and the size of the irradiation spot was 7 mm.

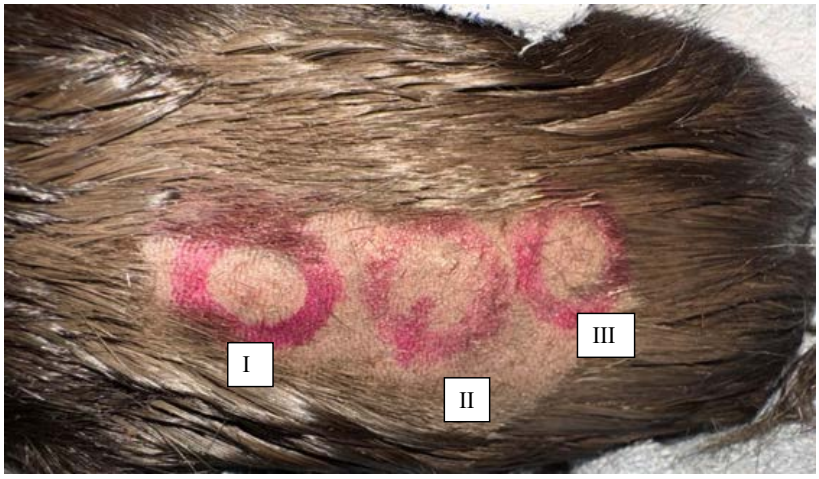
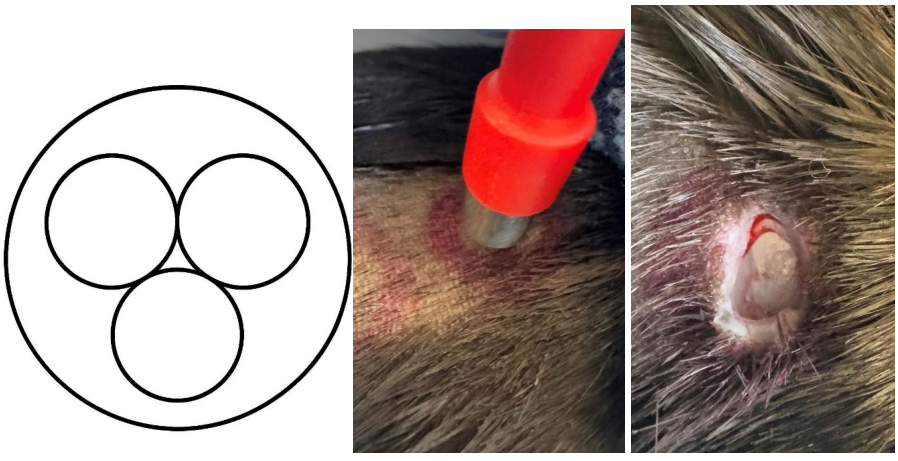


Fig. 2. General view of the marking scheme for future irradiation areas on the skin of an experimental animal

In animals, a punch biopsy of the skin in the laser exposure zone was performed from the three indicated areas – I, II, and III. During the procedure, three fragments of skin were taken together with the platysma, retreating approximately 0.5 cm from the edges of the energy exposure area. The material for histological examination was taken once and synchronously (Fig. 3).



A B C

Fig. 3. Scheme of skin biopsy sampling in experimental animals (A); general view of the punch biopsy stage (B); general view of the skin area after biopsy (C)

The collected material was fixed in 10% aqueous neutral formalin solution for at least 48 hours, after which it was washed, dehydrated and embedded in paraffin according to a standard protocol. Paraffin

sections with a thickness of 5-7 μm were stained with hematoxylin and eosin. Microscopic examination was performed on an OLIMPUS BX 41 light microscope at magnifications of 40, 100, 200 and 400 times. During microscopy, the following morphometric indicators of the dermis were assessed: relative area of stromal edema; relative area of microcirculatory vessels; average number of vessels in the dermis; diameters of capillaries, precapillaries, postcapillaries, as well as arterioles and venules of the dermis; number of inflammatory cells (segmented nuclear leukocytes, plasma cells, monocytic elements, macrophages) per 1 mm²; density of inflammatory cell infiltrate.

To display color images of histological preparations on the screen, a Leadtek WinFast VC 100 video capture card was used. The obtained images were processed, and morphometric measurements and statistical analysis were performed using the Quick PHOTO MICRO 2.3 program (license agreement № 925113924), which provides processing of 2737 pixels.

Statistical data processing was carried out using Microsoft Excel tools and the MS Statistica 10 package.

The study was conducted in compliance with the basic principles of bioethics, in accordance with the Declaration of Helsinki, and approved by the Bioethics Commission of the National Pirogov Memorail Medical University (Protocol № 12 dated May 28, 2025).

Research results and their discussion. Given the capabilities of the Monte Carlo method, which allows us to study random processes through the simulation of real physical phenomena, we proceeded from the tetrahedron model in the analysis of light propagation and energy distribution. Given that each individual «cell» in the model corresponds to the complex geometry of skin vessels, we share the reasonable assumption of a number of researchers that the optical properties of tissues are constant and uniform within each structural component [7].

On this basis, the dorsal dermal chamber (DSC) model was chosen for conceptual justification, which determined the further research strategy and ensured the preservation of the epidermal layer, which partially reflects the histoarchitectonics of the tissues covering the superficial vascular formations. The use of such a model is consistent with Pascal's law, according to which pressure in liquids and gases is transmitted evenly in all directions, confirming the phenomenon of «incompressibility» of the liquid (in our case, blood) [8-10].

In view of this, blood, which constitutes a significant proportion of the volume of any superficial vascular formation, in the process of tumor or malformation progression creates hydrodynamic pressure on the surrounding tissues. This, in turn, causes thinning and gradual structural degradation of the epidermis, which is accompanied by a decrease in melanin content and a reduction in the elements of the skin appendages (hair follicles and glands). This condition significantly reduces the energy losses of laser radiation in the superficial epithelial layer.

According to the authors' conclusions, the energy threshold of vascular bleeding and the nature of the spread of vascular rupture zones closely correlate with purpura caused by hemorrhages. During a laser therapy session, this phenomenon is clinically assessed by the ratio of the area of purpura, i.e.:

$$Sratio. = \frac{Ssq}{\mathrm{H} \times Slsp}, \text{ where (1)}$$

S_{ratio} – ratio of areas of purpura (hemorrhages);
$S_{sq.}$ – total area of purpura after laser therapy session;
N– total number of laser pulses;
S_{lsp} – laser spot area.

The effective and clinically adequate range of Sceq., according to the authors' recommendations, should be assessed according to the degree of recovery one month after the first treatment session. In cases of excessively intense energy exposure, the area of bleeding will also be significantly larger, and the treatment may be accompanied by side effects such as hyperemia, thermal burns, blistering, scarring and/or hypo- or hyperpigmentation. In the absence of side effects after one month, an acceptable level of clinical result corresponds to the limits of Sceq. from >60% to <90%. At the same time, the average value of Sceq. shows a tendency to almost uniform increase with increasing laser irradiation flux, while the optimal value of Sceq. should exceed the level of Sceq. characteristic of clearly positive results and be lower than Sceq. associated with side effects [11-14]. According to the TSFT, clinical efficacy is achieved under conditions of high peak radiation density and short pulse duration, which maximizes the selectivity of the effect. An increase in the diameter of the laser beam spot contributes to better tissue heating, while deeper heating is possible when using lower fluences. Thus, the fluence threshold corresponding to the onset of vascular bleeding (purpura) within the experimental model of the dorsal skin chamber (DSC) is defined as the effective laser density (ELD) [15-17]. However,

the question of the nature of structural changes in the microcirculatory link in different layers of the dermis at the early stages – during the first 1-1.5 hours after a single laser irradiation – remains unresolved. To address this issue, we conducted a macroscopic assessment of the appearance of skin areas after sequential irradiation of three experimental zones: after 30 min. (area I), 60 min. (area II) and 90 min. (area III) (Fig. 4).

Fig. 4. Macroscopic appearance of the skin area after laser irradiation – after 30 min. (area I), 60 min. (area II) and 90 min. (area III)

During the implementation of the experimental study, we took into account that the guinea pig has a histological structure of the skin, which is closest to the human dermis. The outer surface of the skin is represented by a multilayered flat keratinizing epithelium – the epidermis. Under it is a connective tissue base – the dermis, which passes into the subcutaneous tissue (hypodermis) without a sharp boundary. The dermis consists of two types of connective tissue itself, forming the papillary and reticular layers, which do not have a clear boundary between them. The papillary layer, formed by loose fibrous connective tissue, is characterized by uneven expression: in some places it is barely noticeable, while in other areas the surface of the epidermis significantly protrudes, reproducing the contours of connective tissue papillae. The reticular layer is well developed and represented by dense fibrous unformed connective tissue, containing skin appendages – hair and the sebaceous glands associated with them; hair shafts protrude above the surface of the skin. Separate elements of the skin appendages also occur in the hypodermis, to which the reticular layer gradually passes. The basis of the hypodermis is adipose tissue, formed by adipocytes organized into lobules – compact clusters of fat cells of various shapes and sizes. The lobules are separated by thin layers of loose fibrous connective tissue, within which blood vessels and nerves pass [21-25]. On the inner side, the hypodermis is delimited by the platysma (Fig. 5).

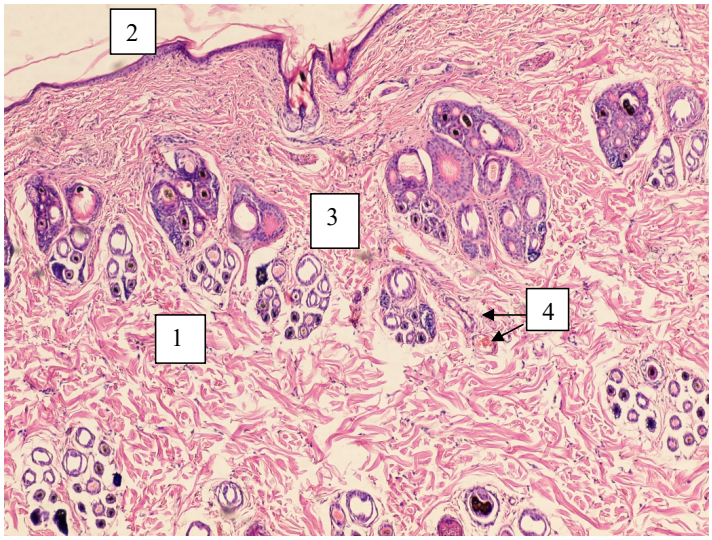


Fig. 5. Intact animal. Normal histological structure of guinea pig skin: 1 – collagen fibers of the dermis; 2 – epidermis; 3 – hair follicles; 4 – microcirculatory vessels. Hematoxylin and eosin staining. Magnification ×100

30 minutes after the start of the experiment, after laser irradiation, noticeable morphological changes were determined in the epidermis layer. Its moderate thickening was observed, caused by mild edema, as well as focal balloon dystrophy of epithelial cells. The area of primary tissue damage was visualized as a thin, barely noticeable strip of fuzzy contours in the slightly edematous dermis. In this area, scattered infiltration by segmented neutrophilic leukocytes (in some places with signs of leukoclasia) was noted, among which there were single lymphocytes and cells of the monocytic series, which formed the so-called reactive zone. Macrophages and histiocytes were found among the cells of the monocytic link. Macrophages had a rounded or irregularly oval shape, varied in size; their nuclei were located eccentrically and differed in the degree of basophilia. The cytoplasm of macrophages was vacuolated and contained pieces of decaying tissues, as well as fragments of dead leukocytes. Histiocytes were localized mainly in the deeper parts of the dermis, had a rounded or elongated shape, their cytoplasm was weakly basophilic, clearly delineated, slightly granular and vacuolated. The nuclei of histiocytes were small, dark, rounded, oval or bean-shaped, with large clumps of chromatin. Moderate edema of the reticular layer of the dermis and subepidermal sections was also detected. Endothelial cells of the vessels of the microcirculatory bed had enlarged, swollen, pale-colored nuclei, some of which were in a state of pyknosis. In general, the structure of the dermis remained sufficiently preserved: fuchsinophilic bundles of collagen fibers of dense fibrous irregular connective tissue were preserved (Fig. 6).

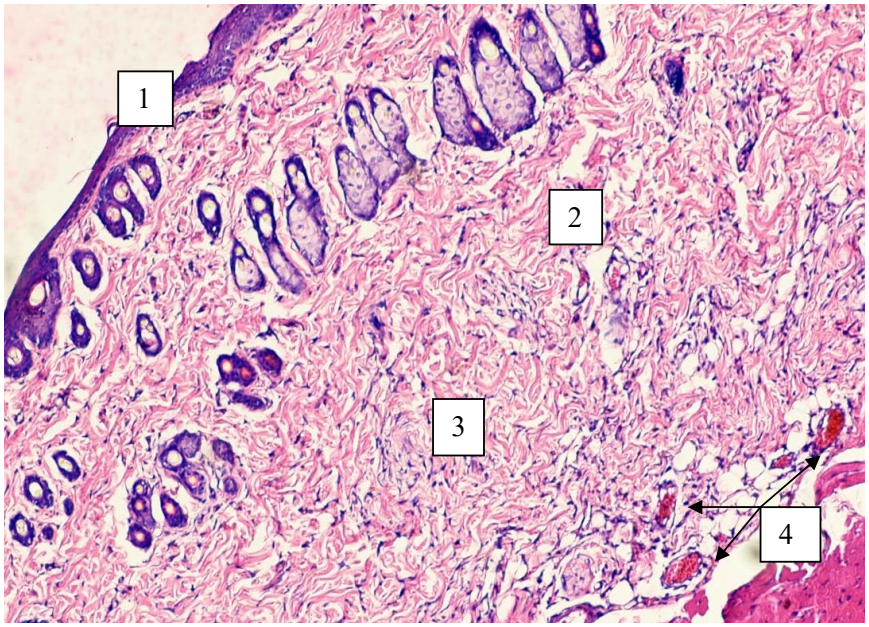


Fig. 6. Skin of an experimental guinea pig 30 minutes after irradiation: 1 – dystrophically altered epidermis of uneven thickness; 2 – slight edema of the dermis; 3 – diffuse inflammatory cell infiltration; 4 – dilated small blood vessels. Hematoxylin and eosin staining. Magnification ×100

60 minutes after the start of the experiment, changes of a mixed nature were detected in the epidermis layer. In some places, its moderate thickening was observed, caused by mild edema and focal balloon dystrophy of epithelial cells. In other areas, on the contrary, thinning and compaction of the epidermis was observed due to its local coagulation. The spread of stromal edema to the superficial layers of the skin was also noted, which indicated the development of a pronounced vascular reaction. The vessels of the deep layers of the dermis were full of blood; perivascular edema and erythrocyte stasis in the vessels of the microcirculatory bed were preserved. Compared with the previous time interval, the changes spread deeper into the superficial parts of the skin, and edema in the dermis became more noticeable. The vascular reaction was manifested by the fullness of small-caliber vessels, which caused an increase in the relative area of the dermal vessels and the average diameter of the vessels. Minor perdiapedic hemorrhages were observed. Reactive inflammatory infiltration was moderate. The cellular composition of the infiltrate was dominated by segmented neutrophilic leukocytes (sometimes with signs of leukoclasia), among which lymphocytes and macrophages were found in small numbers. Collagen fibers of dense fibrous irregular connective tissue of the dermis were preserved, although they were slightly damaged (Fig. 7).

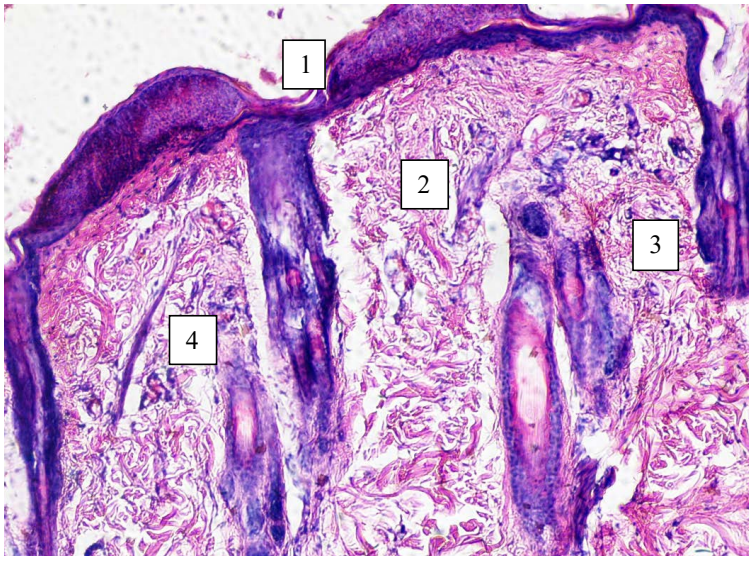


Fig. 7. Skin of an experimental guinea pig 60 minutes after irradiation: 1 – dystrophically altered epidermis of uneven thickness with foci of coagulation; 2 – moderate edema of the dermis; 3 – diffuse inflammatory cell infiltration; 4 – dilated small blood vessels. Stained with hematoxylin and eosin. Magnification ×100

90 minutes after irradiation, a significant disruption of the organization of skin structures is noted, which is due to the formation of foci of coagulation changes and more intense inflammatory infiltration with the participation of segmented neutrophilic leukocytes. Lymphohistiocytic cells are less common. The vessels of the hemomicrocirculatory bed of the dermis demonstrate signs of uneven blood supply. More pronounced edema of the dermis is observed. The processes of leukopedesis are more intense. In the dermal layer, fuchsinophilic strands of collagen fibers of ordered fibrous connective tissue with signs of coagulation are detected. The vascular reaction of the skin is manifested by paretic expansion, hyperemia and thrombus formation in medium and large caliber vessels, which causes a noticeable increase in the relative area of the vascular bed of the dermis and the average diameter of the vessels. The epidermis in some areas is compacted, thinned or undergoes desquamation (Fig. 8).

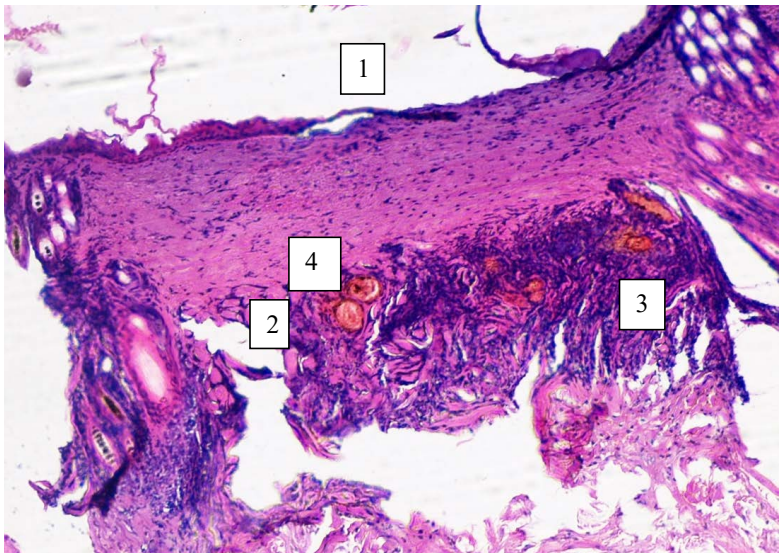


Fig. 8. Skin of an experimental guinea pig 90 minutes after irradiation: 1 – dystrophically altered epidermis of uneven thickness with foci of coagulation and desquamation; 2 – pronounced edema of the dermis; 3 – diffuse significant inflammatory cell infiltration; 4 – significantly dilated full-blooded and thrombosed small vessels. Stained with hematoxylin and eosin. Magnification ×100

The second stage of the experiment was a comparative analysis of the morphometric characteristics of the dermis established by us. 30 minutes after the experimental exposure to laser radiation, the relative cross-sectional area of the vessels of the microcirculatory bed was 8.14 ± 0.12%, and

their average diameter was 13.24 ± 0.35 μm. Single inflammatory cell elements of various types (segmented neutrophils, plasma cells, mononuclear cells similar to blood monocytes, and macrophages) were recorded in the dermis. The quantitative indicators were: segmented leukocytes – 9 cells/ mm², plasma cells – 5 cells/mm², mononuclear cells of the monocytic type – 8 cells/mm², macrophages – 3 cells/mm². The total density of inflammatory cell infiltrate was 25 cells/ mm². The vessels of the dermis were dilated, filled with blood, the endothelium was swollen, perivascular edema was noted in the dermis. The phenomenon of erythrocyte sludge was observed in some vessels. No signs of thrombosis or hemorrhage were recorded. The diameter of the vessels reached 20.18 ± 0.61 μm, the average number was 17.2 ± 0.27. The combination of these changes characterized minor disorders of microcirculation in the irradiated tissues. The relative area of stromal edema was 14.03 ± 0.52%, which reflected a weakly expressed vascular response and a moderate increase in the permeability of their walls in this time interval after energy exposure.

60 minutes after the start of the experiment, the relative area of the vessels of the microcirculatory bed increased to 9.15 ± 0.16%, and the average diameter was 15.45 ± 0.46 μm. In the dermis, a greater number of inflammatory cell elements was recorded than after 30 minutes. The indicators were as follows: segmented leukocytes – 13 cells/mm², plasma cells – 9 cells/ mm², mononuclear cells of the monocytic type – 15 cells/ mm², macrophages – 6 cells/mm². The total density of the infiltrate reached 43 cells/mm². The vessels of the dermis were moderately dilated, full-blooded, the endothelium remained swollen, moderate dermal edema was noted. In some vessels, the phenomenon of erythrocyte sludge was determined. No signs of thrombosis were detected. Perivascular perdiapedic hemorrhages were observed. The average diameter of arterioles and venules was 22.86 ± 0.64 μm, their average number was 18.9 ± 0.31. Such changes indicated a moderate violation of microcirculation. The relative area of stromal edema reached 15.98 ± 0.59%, which corresponded to a moderately pronounced vascular response and increased permeability of the vascular walls in the affected area.

90 minutes after the start of the experiment, the relative area of the vessels of the microcirculatory bed of the dermis reached 10.12 ± 0.31%, and the average diameter was 16.12 ± 0.41 μm. Areas of coagulation of collagen fibers and a greater number of inflammatory cells than after 60 minutes were detected in the dermis. The number of segmented leukocytes was 17 cells/ mm², plasma cells – 14 cells/ mm², monocyte-like mononuclear cells – 18 cells/mm², macrophages – 9 cells/mm². The total density of the infiltrate reached 58 cells/mm². The vessels of the microcirculatory bed were significantly dilated, sharply full-blooded, the endothelium was markedly edematous, and intense edema was determined in the dermis. Erythrocyte sludge was recorded, and in individual vessels thrombosis. Perivascular focal hemorrhages were observed. The average diameter of arterioles and venules reached 23.45 ± 0.54 μm, their number was 20.78 ± 0.37. The set of changes indicated significant disorders of microcirculation in the irradiated area of the skin. The relative area of stromal edema was 16.87 ± 0.55%, which reflected an intense vascular reaction and a significant increase in the permeability of the vascular walls in the areas of damage to the dermis.

The obtained morphometric data are summarized in a table in order to further objectify the analysis of the dynamics of their changes and establish the nature of the relationships (Table 3).

Table 3

Dynamics of changes in morphometric parameters of the study in experimental animals

Morphometric indicators	Time of determination of morphometric indicators				
	Through 30 minutes	Through 60 minutes	p*	Through 90 minutes	p*
Relative area of stromal edema, (%)	14.03±0.52	15.98±0.59	<0.05	16.87±0.55	<0.05
Relative area of microcirculatory vessels, (%)	8.14±0.12	9.15±0.16	<0.05	10.12±0.31	<0.05
Average number of dermal vessels, (pcs.)	17.2±0.27	18.9±0.31	<0.05	20.78±0.37	<0.05
Average diameter of microcirculatory vessels, (μm)	13.24±0.35	15.45±0.46	<0.05	16.12±0.41	<0.05
Average diameter of arterioles and venules of the dermis, (μm)	20.18±0.61	22.86±0.64	<0.05	23.45±0.54	<0.05
Number of inflammatory cellular elements of the dermis in 1 mm²: segmented leukocytes	9	13		17	
plasma cells	5	9		14	
blood monocytes	8	15		18	
macrophages	3	6		9	
Density of inflammatory cell infiltrate, (mm2)	25	43		58	

* – significance of the difference relative to the indicators 30 minutes after skin irradiation

Analysis of the relative area of microcirculatory vessels and the average number of dermal vessels revealed a practically similar directly proportional tendency to increase over time. In addition, the study of the dynamics of changes in the relative area of stromal edema showed that it correlated to a greater extent with the corresponding values of the relative area of dermal vessels (Fig. 9).

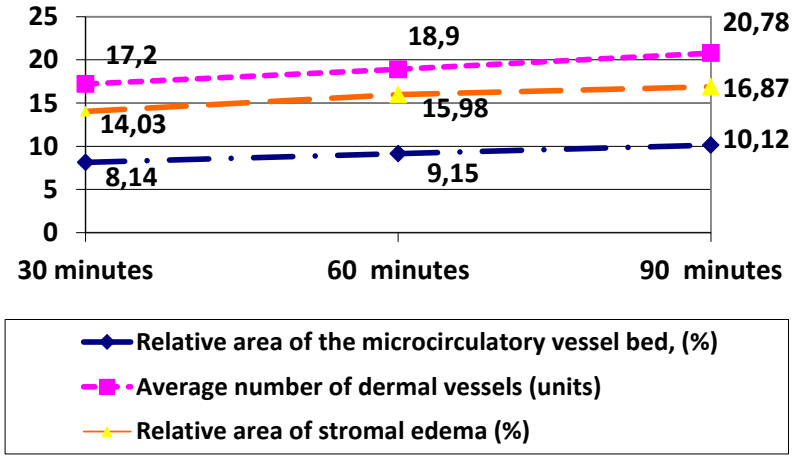


Fig. 9. Linear graphs of the dependence of the values of the relative area of microcirculatory vessels and the average number of dermal vessels on the relative area of stromal edema

Comparing the values of the average diameter of the microcirculatory vessels and the average diameter of the arterioles and veins of the dermis, it was found that, depending on the terms of the experiment, there was an almost directly proportional tendency to their simultaneous growth. At the same time, the reference time indicators of the relative area of stromal edema also had a positive tendency to increase (Fig. 10).

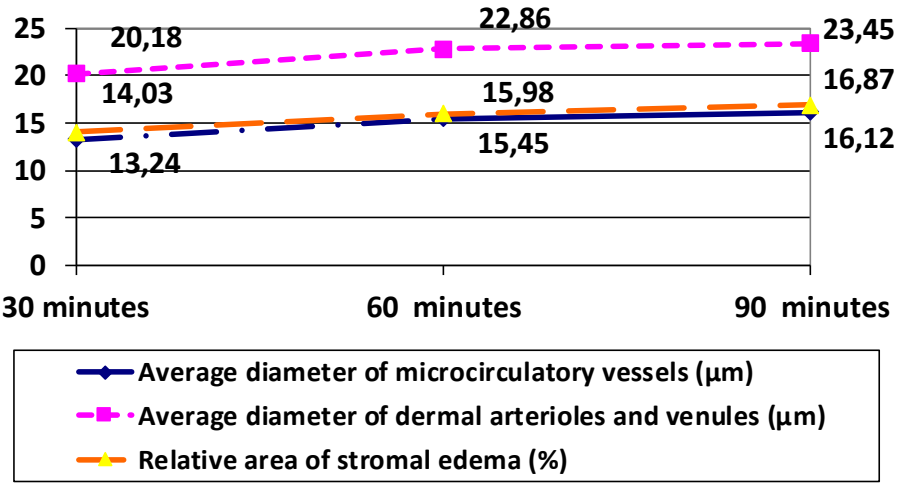


Fig. 10. Linear graphs of the dependence of the average diameter of the microcirculatory vessels and the average diameter of arterioles and venules on the relative area of stromal edema

Therefore, the increase in the average diameter of the microcirculatory vessels and, accordingly, the average diameter of the arterioles and veins of the dermis caused an increase in the relative area of stromal edema over the course of the experiment. At the same time, the magnitude of the relative area of stromal edema is largely determined by the dependence on the average diameter of the microcirculatory vessels, which indicates a more pronounced energy effect of laser irradiation of the skin on the peripheral part of its blood circulation, causing irreversible destructive processes in it (formation of microthrombi, obliteration of the lumen) under the constant influence of pressure, due to edema, on the vascular wall from the outside.

Analysis of the dynamics of the relative area of the microcirculatory vessels and the average diameter of the microcirculatory vessels determined the existence of almost directly proportional dynamics depending on the duration of the experiment. Also indicative, in our opinion, was the fact that the relative area of stromal edema, at all times of the experimental study, was more closely correlated with the values of the average diameter of the microcirculatory vessels (Fig. 11).

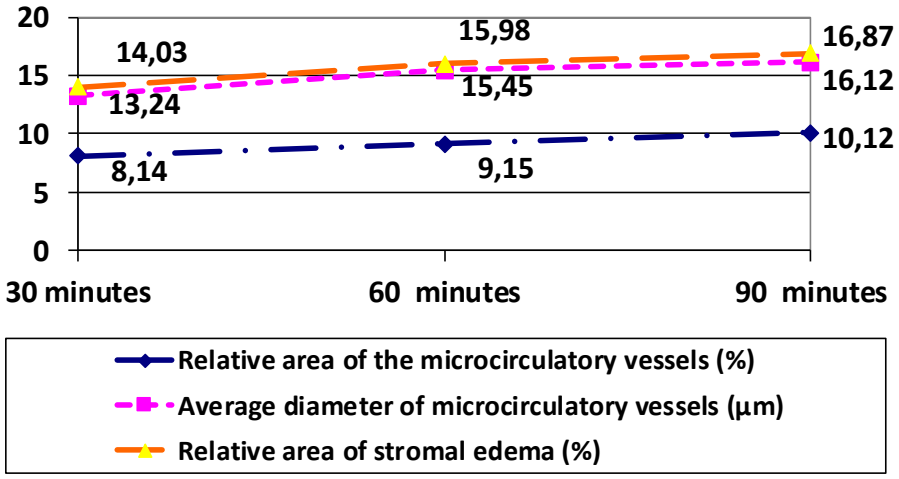


Fig. 11. Linear graphs of the dependence of the relative area of microvascular vessels and the average diameter of microvascular vessels on the relative area of stromal edema

Comparison of the values of mutual growth of the average diameter of arterioles and venules of the dermis and the average number of vessels of the dermis showed the existence of almost directly proportional dynamics in accordance with the terms of the experiment. However, in our opinion, it is important that there is no close correlation with the indicators studied, the values of the relative area of stromal edema, although the latter had a closer relationship, in terms of its values, with the average number of vessels of the dermis (Fig. 12).

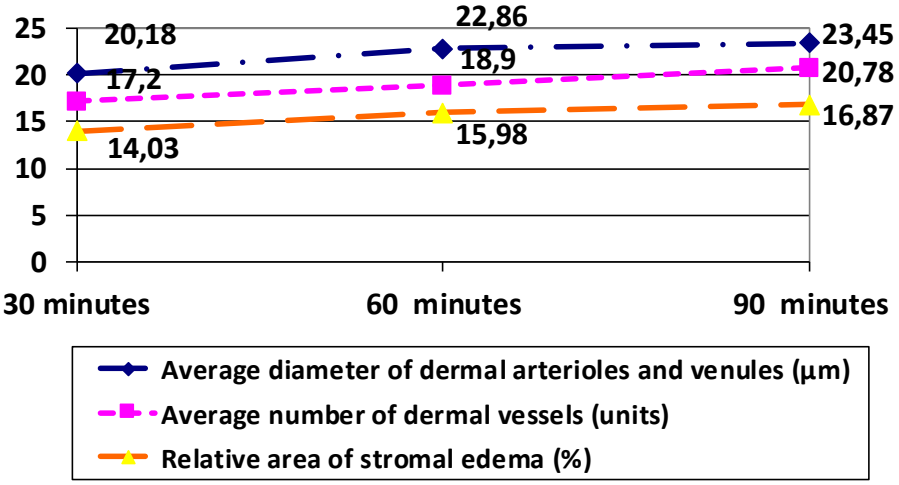


Fig. 12. Linear graphs of the dependence of the average diameter of arterioles and venules of the dermis and the average number of vessels of the dermis on the relative area of stromal edema

Thus, the analysis of the dynamics of morphometric indicators of the microcirculatory link of the dermis revealed a certain linear relationship between them and the size of the relative area of stromal edema, which is one of the key components of skin purpura in all studied time intervals of the experiment. At the same time, the relative area of stromal edema showed a tendency to increase, despite the gradual decrease in visible manifestations of purpura during the observation period. This nature of the external course of purpura after a single laser irradiation of the skin turned out to be inversely related to the severity and prevalence of morphological changes in deeper dermal structures. Thus, 60 minutes after irradiation, more intense, compared to a 30-minute interval, pleural and perivascular edema were observed in combination with erythrocyte stasis in the vessels of the microcirculatory bed. This was accompanied by minor prediapedic hemorrhages, the development of reactive inflammatory infiltrate and damage to connective tissue structures. Even more significant and profound morphological changes were detected 90 minutes after laser exposure. They were manifested by foci of coagulation in the structure of connective tissue, significant inflammatory infiltration with the participation of segmented neutrophils, pronounced edema of deep dermal layers, uneven blood supply to the vessels of the microcirculatory bed and increased leukopenia. The epidermis was represented by areas of local compaction, thinning and focal desquamation. The vascular reaction

of the skin was characterized by paralytic dilatation, hyperemia and thrombosis of medium and large caliber vessels, which contributed to an increase in their relative area and, accordingly, an increase in the area of stromal edema during the pathological process.

Conclusions. 1. The phenomenon of skin purpura after a single laser irradiation can and should be considered as a clinically significant marker of the effectiveness of selected energy parameters of exposure. 2. Comparison of the dynamics of purpura involution with the morphological and morphometric characteristics of vascular changes in all dermal layers indicates the rapid spread of pathological processes in them against the background of increasing severity of stromal edema, which progresses towards the deeper layers of the dermis already in the early stages after irradiation. 3. The obtained experimental data confirm the possibility of the formation of cyclic pathological changes in the structural components of the dermis, primarily in the microcirculatory and vascular bed, after each subsequent session of laser therapy within the framework of individual treatment regimens for vascular lesions of the skin.

Prospects for further research. Further research should focus on an in-depth investigation of the cumulative and delayed effects of repeated laser therapy sessions on the dermal microcirculatory network, taking into account various irradiation modes, laser types, and wavelengths. Establishing clear morphological and morphometric criteria for the safety and efficacy of laser exposure appears promising, as it may contribute to the optimization of individualized treatment protocols for cutaneous vascular lesions, particularly in pediatric practice. Special attention should be paid to correlating clinical manifestations with deep structural changes in the dermis, as well as to studying the mechanisms of connective tissue regeneration following laser-induced injury.

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EXPERIMENTAL EVALUATION OF LASER IRRADIATION EFFECTS ON THE DERMAL MICROCIRCULATORY SYSTEM

Abstract. The high efficacy and safety of laser therapy allow for initiating treatment at early stages of diseases in patients of various age groups, including pediatric practice. This approach enables not only a direct impact on the pathological focus but also reliable prevention of potential complications, emphasizing the importance of the studied problem. The inherent advantages of laser radiation in treating cutaneous vascular lesions require further detailed investigation to determine the optimal conditions for its use – selection of laser type, rational wavelengths, power, and other biotechnical characteristics of irradiation.
The aim of the study is to investigate pathological changes in dermal microcirculatory vessels after single exposure to laser irradiation with standard power and duration parameters, and to determine their relationship with involutive processes of skin purpura.
Material and methods. An analysis was performed of histological changes and tissue reactions of the skin following sequential laser irradiation at 30 minutes (site № 1), 60 minutes (site № 2), and 90 minutes (site № 3). The experiment was conducted on guinea pigs weighing 350.0-400.0 g and aged 6-8 weeks, selected due to the morphological similarity of their skin structure to human skin. Analysis of the dynamics of morphometric parameters of the dermal microcirculatory network revealed a linear relationship between these parameters and

the relative area of stromal edema – a key component of skin purpura – at all studied time points. The area of dermal stromal edema tended to increase despite a decrease in visible manifestations of purpura. An inverse relationship was observed between the clinical dynamics of superficial purpura manifestations and the severity of morphological changes in the deeper dermal layers. Sixty minutes after irradiation, marked hyperemia, perivascular edema, and erythrocyte stasis in the microcirculatory vessels were observed compared with the 30-minute interval, alongside moderate peridiapedetic hemorrhages, reactive inflammatory infiltration, and damage to structural components of connective tissue.
Conclusions. The appearance of skin purpura after a single laser irradiation session can be considered a clinically significant marker of the adequacy of selected energy parameters. Comparison of purpura involution dynamics with morphological and morphometric indicators of vascular changes in all dermal layers indicates rapid propagation of pathological processes against a background of increasing stromal edema area at early stages post-irradiation. The obtained results confirm the potential for cyclic pathological changes in dermal structural components, primarily the microcirculatory and vascular networks, following each subsequent laser therapy session within an individualized treatment program for cutaneous vascular lesions.
Key words: dermis, vascular formations, skin, treatment, experiment, skin morphology, pathological changes, microcirculatory system.

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