

морфогенез надпочечников; при этом наиболее активная перестройка идентифицирована в пучковой зоне коры. На 1-е сутки постнатального онтогенеза на фоне гипотиреоза выявлено задержку развития мозгового вещества, тогда как гипертиреоз сопровождался угнетением пролиферативной активности клеток клубочковой зоны надпочечников. На 10-е сутки постнатального развития на фоне тиреоидного дисбаланса задокументировано усиление процессов пролиферации и апоптоза в составе коры в сочетании с угнетением интенсивности обоих процессов в мозговом веществе надпочечников.

Ключевые слова: крысы, онтогенез, надпочечники, материнский гипо- и гипертиреоз, иммуногистохимическое исследование.

rearrangement was identified in the puchous zone of the cortex. On the first day of postnatal ontogenesis, hypothyroidism was associated with a delay in the development of the medulla, while hyperthyroidism was accompanied by inhibition of the proliferative activity of the cells of the glomerular zone of the adrenal glands. On the 10th day of postnatal development against the background of thyroid imbalance, there is a documented increase in the processes of proliferation and apoptosis in the composition of the cortex in combination with the inhibition of the intensity of both processes in the medulla of the adrenal glands.

Key words: rats, ontogenesis, adrenal glands, maternal hypo-and hyperthyroidism, immunohistochemical research.

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DYNAMICS OF HISTOCHEMICAL CHANGES IN THE SKIN OF RATS WITHIN A MONTH AFTER THE BURNING OF II-III DEGREES ON THE BACKGROUND OF THE INJECTION FIRST 7 DAYS HAES-LX-5% SOLUTION

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The purpose of the work was to study the state of elastic, collagen fibers, glycoproteins and glycosaminoglycans in the intercellular substance of the dermis of male rats 1, 3, 7, 14, 21 and 30 days after the burning of II-III degrees on the background of injecting HAES-LX-5% solution in first 7 days. During 1-3 days after a thermal 2-3 degrees injury and the injection of HAES-LX-5% solution, the reorganization of the intercellular substance of the dermis appears less damaging to the fibrous structures of its papillary and reticular layers. Histochemically amorphous connective tissue substance is rich in glycoproteins, the content of glycosaminoglycans is still small. When HAES-LX-5% injected after 7 and 14 days of the experiment, the Van Gieson-Weigert method allowed to see renewal of collagen fibers in the boundary regions of the affected skin, located mainly wavelike, like thin beams. In the late stages of the experiment (after 21 and 30 days), the active course of regeneration contributes to a significant normalization of the fibrous structures of the dermis. In the intercellular substance of the dermis, the content of amorphous compounds is normalized, a moderate "Hale"-positive coloration is histochemically determined, indicating a decrease in the number of sulfated glycosaminoglycans, and the content of neutral glycosaminoglycans and glycoproteins is reduced, their PAS-positive properties are moderately expressed.

Key words: thermal burns of the skin, infusion solutions, male rats, histochemical studies.

The study is a fragment of the research project "Structural changes in the skin after thermal burn and their correction by colloid-hyperosmolar solutions (experimental research)", state registration No. 0118U003192.

Despite some progress achieved in combustiology, the mortality rate for burn disease remains high and reaches 80-90%. Severe thermal trauma is considered a serious damaging factor for the whole body (violation of hemodynamics, function of the gastrointestinal tract, liver, kidneys, metabolism, immunity, etc.) [8, 13].

Critical fluid loss is the biggest problem that occurs after burns. It is established that already in the first minutes after receiving an injury there is a change in the rheological properties of the blood. Effective fluid renewal is one of the cornerstones of modern treatment of burn injury [5, 9].

Restoration of blood volume means the prevention and correction of water-electrolyte, and metabolic disorders. It is recommended to use complex crystalloid solutions maximally close to the electrolyte composition of blood plasma (Hartmann solution, lactosol, etc.). A study on healthy volunteers showed that only about 30% of the administered crystalloid remains in the target, that is, the intravascular space. Depending on pathophysiology, in order to achieve comparable volumetric effects, it may be necessary to significantly increase the amount of crystalloid inputs compared with colloids [14].

Optimal is the use of colloidal volumetric and plasma substitute environments, a number of properties which allows them to be widely used in the clinic: a good exchangeable capacity, the absence of antigenic properties and toxicity, destruction and complete excretion from the body. These drugs do not have a negative effect on coagulation of blood and do not cause allergic reactions. Colloidal solutions based on gelatin or hydroxyethyl starch are mainly contained in the intravascular space, while infusion with crystalloid cells primarily affects the extravascular space [4, 10].

In accordance with the latest recommendations for treatment of burn disease, in addition to the use of colloidal solutions should be supplemented with the help of polyhydric alcohols [12]. Due to the insufficiency of insulin in patients in critical condition, large amounts of glucose will be metabolized to lactate, thereby increasing lactic acidosis. A possible way out of this situation is the use of polyatomic alcohol xylitol, the metabolism of which passes without the participation of insulin. In addition, he has an adequate detoxification, rheological, anti-edematous, antishock action [3].

The combination of the qualities of the above-mentioned groups of drugs in the complex preparation may become optimal in the pathogenetic treatment of burn disease. The drug that meets the requirements is currently being developed. This is a new promising colloid-hyperosmolar solution based on hydroxyethyl starch HAES-LX-5% and polyatomic xylitol alcohol, the intrinsic component of sodium lactate - 1,5%, sodium chloride - 0,8%, potassium chloride - 0,03%, calcium chloride - 0.02%, magnesium chloride - 0.01%, ionic composition of the preparation: Na + 270.7 mmol/l, K + 4.0 mmol/l, Ca ++ 1.8 mmol/l, Mg ++ - 1.1 mmol/l, Cl⁻ - 146.6 mmol/l, whose study on the action of experimental animals is actively carried out by scientists [1, 6].

The purpose of the study was to investigate the state of elastic, collagen fibers, glycoproteins and glycosaminoglycans in the intercellular substance of the dermis of male rats 1, 3, 7, 14, 21 and 30 days after the burning of II-III degrees on the background of the injection first 7 days HAES-LX- 5%.

Material and methods. The study of structural changes in the skin after its burn injury on the background of the introduction first 7 days HAES-LX-5% solution was performed on laboratory white rats, males weighing 150-160 g. Rats were divided into 2 groups, which were previously, under the conditions of propofol anesthesia, catheterization of the femoral vein and depilation of the lateral surfaces of the trunk of the rats were performed.

Group 1 - animals without thermal trauma of the skin which once a day for the first 7 days, intravenous infusion of HAES-LX-5% solution in a dose of 10 ml per kg was performed.

Group 2 - animals that were also injected once daily with the first 7 days of infusion of HAES-LX-5% solution, at a dose of 10 ml per kg after 2-3 degree skin burns, with a total area of 21-23% of the body surface of the rat.

Burn skin damage was caused by applying to the shaved lateral surfaces of the trunk of the rats of four copper plates (two plates on each side, the surface area of each plate was 13.86 cm²) which were preheated for 6 minutes in water at a constant temperature of 100 °C. A similar burn injury was sufficient for the formation of 2-3 degree burns and the induction of a shock state of moderate severity [29], which was confirmed by collaborative research with a team of research center of the National Pirogov Memorial Medical University, Vinnytsya [7].

For the study of histochemical changes in the skin after severe thermal trauma, the animals were withdrawn from the experiment by decapitation using guillotine in 1, 3, 7, 14, 21 and 30 days, according to the current classification, according to the stages of burn disease: respectively, shock, early and late toxemia and septicotoxemia.

Before the selection of material examined the burn surface, noted the features of burn scab and the presence or absence of purulent complications in the area of the wound. After decapitation, under propofol anesthesia, for 5 minutes, we took pieces of the skin from the marginal part of the wound to fix the material prior to the development of posthumous changes. Sections were fixed in 10% formalin solution, while the exposure time did not exceed 1-2 days. The applied fixing solution prevents the autolysis process and stabilizes the cells and tissues for their further processing and use in coloring procedures. Subsequently, dehydration was carried out in spirits of increasing concentration and poured into paraffin blocks. Received on the sledge microtome MS-2 sections 5-6 microns thick stained with hematoxylin and eosin; Weigert's elastic stain and dyed with picric acid by Van Gieson's method (for the detection of elastic and collagen fibers); the PAS + Hale reaction was performed by the Moury method (detection of glycoproteins and glycosaminoglycans in the intercellular substance of the connective tissue). Histochemical slides were investigated in a light microscope of Leitz Laborlux S.

Results of the study and their discussion. In previous studies, O.Ye. Maievskyi and his colleagues [11] studied in detail the state of elastic and collagen fibers, as well as glycoproteins and glycosaminoglycans in the intercellular substance of the dermis of rats, 1, 3, 7, 14, 21 and 30 days after the burn of II-III degrees, with the introduction first 7 days 0,9% NaCl solution.

It should be noted that during the histochemical studies of rats skin in the control group (animals without thermal trauma of the skin, for which first 7 days were injected HAES-LX-5% solution), no discrepancies were found in the state of elastic and collagen fibers, as well as glycoproteins and glycosaminoglycans in the intercellular substance dermis, compared to those described in previous studies when administered to intact animals the first 7 days of 0.9% NaCl solution [11].

When applying the HAES-LX-5% solution in the early stages of the experiment on the periphery of the wound, microscopically observed collagen fibrils that are placed singly or locally homogenized, elastic fibers are not detected (fig. 1 A). This picture shows that collagen and elastin were denatured in large quantities, and protein fibers were transformed into inanimate "ropes".

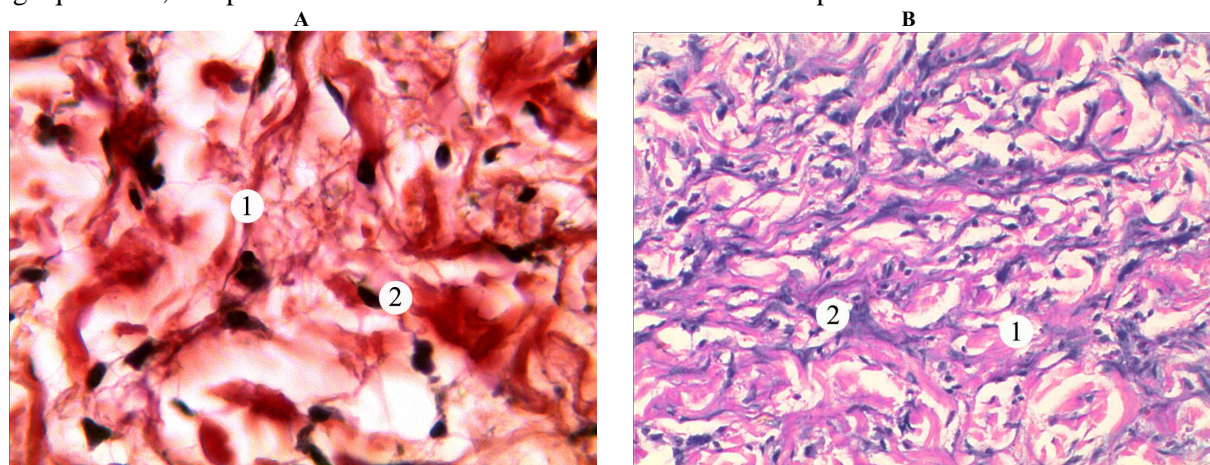


Fig. 1. **A.** Histological condition of the skin derma of the animal 3 days after burn injury by the correction of HAES-LX-5%. Damaged collagen fibers (1), fibroblast (2). Van Gieson's-Weigert's staining. x 600. **B.** Histochemical organization of skin derma of the animal 3 days after burn injury by the correction of HAES-LX-5%. Well expressed PAS - (1) and moderate "Hale"-positive (2) coloration of the connective tissue. Staining by the Moury method. x 200.

Histochemically, 1 and 3 days after burn injury, in the area of correction in the area surrounding the wound, a moderate "Hale"-positive color of the connective tissue and its well-expressed PAS-positive properties (fig. 1 B) is observed, which gives grounds to believe that the amount of glycoproteins increases, and glycosaminoglycans break up. That is, the process of regeneration has not yet begun and dominated by the phenomena of proteolysis and phagocytosis [2].

7 and 14 days after a thermal trauma when the HAES-LX-5% solution is corrected in the reticular layer of the dermis, the formation of collagen fibers with their characteristic wavelike shape and different thickness (fig. 2 A) is observed, indicating that the reparative process runs more intensively, forming a regenerate zone.

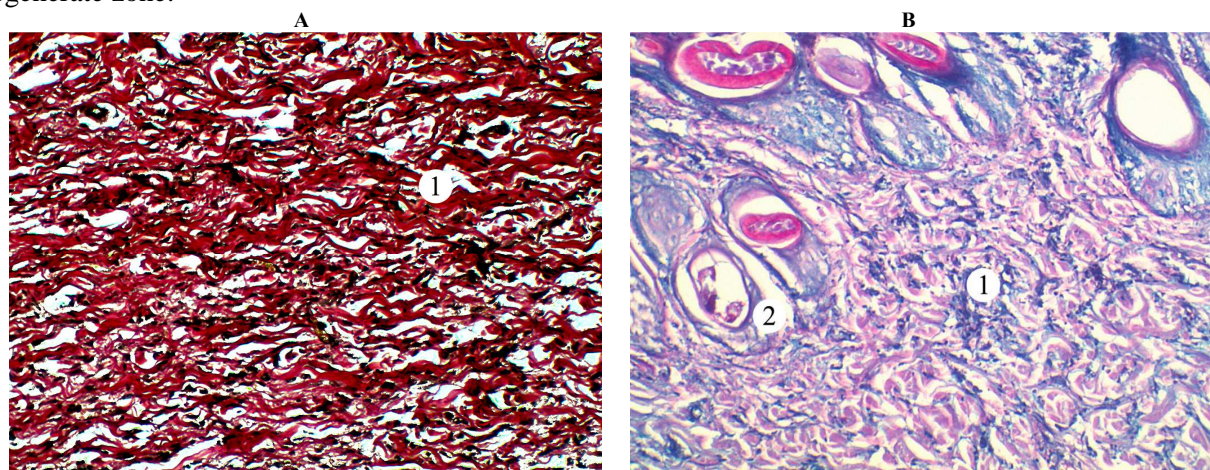


Fig. 2. **A.** Histological condition of the connective tissue of the area of the skin of the animal 14 days after burn injury by the correction of HAES-LX-5%. Different thickness of collagen fibers (1). Van Gieson's-Weigert's coloring. x 200. **B.** Histochemical organization of animal skin 14 days after thermal trauma under conditions of correction by HAES-LX-5%. Bright "Hale"-positive sour glycosaminoglycans (1), appendages of the skin (2). Coloring by the Moury method. x 200.

The use of the histochemical method in these terms of the experiment showed an increase in the content of sulfated glycosaminoglycans, manifested by a pronounced "Hale"-positive color. The PAS-positive properties of the connective tissue are less pronounced in comparison with the early terms of the experiment (fig. 2 B), which indicates the collapse of the collagen and elastin destruction, the increase in content and redistribution of glycosaminoglycans in the intercellular substance of the dermis.

In the later stages of the experiment, when HAES-LX-5% correction was performed on histological preparations, the presence of well-formed collagen and thin elastic fibers was determined by Van Gieson's-Weigert's method. Fibrous structures have a mainly horizontal direction and a wave-like form (fig. 3 A).

Consequently, in the use of the drug, there is a significant reorganization and adjustment for the regeneration of all intracellular organelles that carry out the synthesis, accumulation and removal of collagen in the intercellular space. The burn area begins to perform barrier functions, since it consists of longitudinally located thin collagen fibers connected with proteoglycans [15].

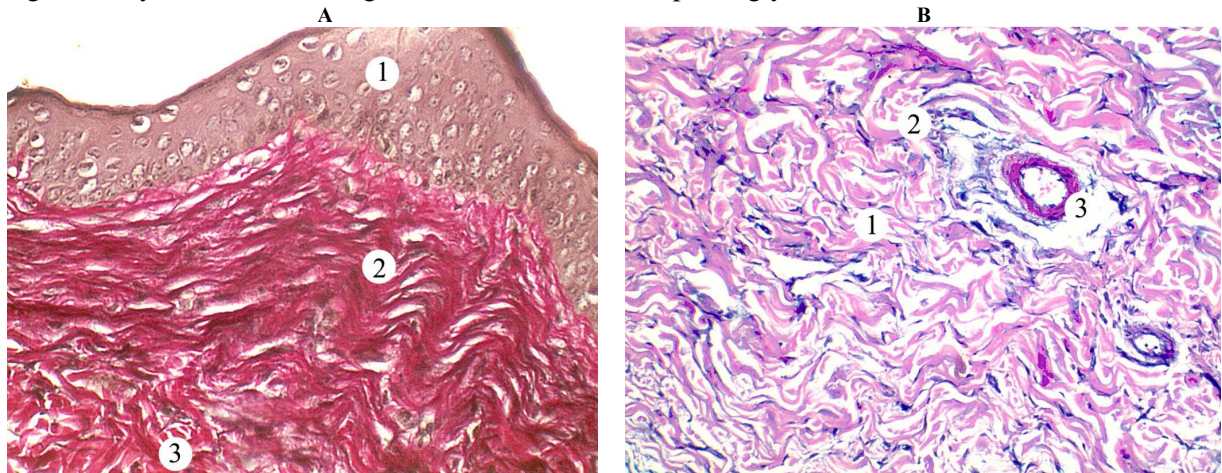


Fig. 3. **A.** Histological condition of animal skin 30 days after thermal trauma under conditions of correction by HAES-LX-5%. Epidermis (1), collagen (2) and elastic (3) fibers. Van Gieson's-Weigert's coloring. x 300. **B.** Histochemical state of the reticular layer of dermis of the skin in the area of the wound 30 days after the thermal trauma under the correction of HAES-LX-5%. Moderately expressed PAS - (1) and "Hale" - (2) positive properties of the intercellular substance of the dermis, the blood vessel (3). Coloring by the Moury method. x 200.

Histochemical studies of the area of skin lesions in 21 and 30 days in the context of HAES-LX-5% correction found a decrease in the number of sulfated glycosaminoglycans among the components of the intercellular substance of the dermis, as compared to the previous terms of the experiment, as evidenced by the moderate "Hale"-positive coloration. The number of neutral glycosaminoglycans and glycoproteins is also reduced, their PAS-positive properties are moderately expressed (fig. 3 B). This suggests slowing down and completing the reparation processes.

It should be noted that the results of microscopic studies in the late stages of the experiment using the HAES-LX-5% solution are close to those obtained in the study of similar indices in the intact group of animals [11].

In the last decade, the use of HAES-LX-5% solution for skin burns has been intensively introduced into practice. Taking into account the data on the positive effect of the drug on the morpho-functional status of the tissues of the lung, small intestine, kidney, spleen [1, 6] in the experiment, one can assume a similar positive result in the skin.

The results of using the HAES-LX-5% solution in rats with dermal burns were first analyzed from the point of view of the peculiarities of pathological changes in the zone of thermal skin lesions at different stages of the wound process. The combined assessment of the results of treatment of dermal burns compared with intact animals [11] suggests the benefits of using this drug.

Conclusions

1. 1 and 3 days after a thermal injury 2-3 degrees and injection of the HAES-LX-5% solution, the reorganization of the intercellular substance of the dermis appears less damaging to the fibrous structures of its papillary and reticular layers. Histochemically amorphous connective tissue substance is rich in glycoproteins, the content of glycosaminoglycans is still small.

2. When HAES-LX-5% injected after 7 and 14 days of the experiment, the Van Gieson-Weigert method allowed to see renewal of collagen fibers in the boundary regions of the affected skin, located mainly wavelike, like thin beams. In the late stages of the experiment (after 21 and 30 days), the active course of regeneration contributes to a significant normalization of the fibrous structures of the dermis. In the intercellular substance of the dermis, the content of amorphous compounds is normalized, a moderate "Hale"-positive coloration is histochemically determined, indicating a decrease in the number of sulfated glycosaminoglycans, and the content of neutral glycosaminoglycans and glycoproteins is reduced, their PAS-positive properties are moderately expressed.

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Реферати

ДИНАМІКА ГІСТОХІМІЧНИХ ЗМІН В ШКІРІ ЩУРІВ ПРОТЯГОМ МІСЯЦЯ ПІСЛЯ ОПІКУ ІІ-ІІІ СТУПЕНЯ НА ФОНІ ВВЕДЕННЯ ПЕРШИХ 7 ДІБ РОЗЧИНУ HAES-LX-5%

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Метою роботи було вивчити стан еластичних, колагенових волокон, глікопротеїнів і глікозаміногліканів у міжклітинній речовині дерми щурів-самців через 1, 3, 7, 14, 21 та 30 діб після опіку ІІ-ІІІ ступеня на фоні введення перших 7 діб розчину HAES-LX-5%. Через 1 та 3 доби після термічної травми 2-3 ступеня за умов застосування розчину HAES-LX-5% реорганізація міжклітинної речовини дерми проявляється меншим пошкодженням волокнистих структур її сосочкового і сітчастого шарів. Гістохімічно аморфна речовина сполучної тканини багата на глікопротеїни, вміст глікозаміногліканів ще невеликий. При введенні HAES-LX-5% через 7 та 14 діб досліду при забарвленні за методом Ван Гізона – Вейгерта у сполучній тканині у крайових ділянках ураженої шкіри спостерігається оновлення колагенових волокон, вони переважно розташовані хвилеподібно, тонкими пучками. У пізні терміни досліду (через 21 та 30 діб) активний перебіг регенерації сприяє значній нормалізації волокнистих структур дерми. У міжклітинній речовині дерми нормалізується вміст сполук аморфної речовини, гістохімічно встановлено помірно "Хейл"-позитивне забарвлення, що свідчить про зменшення кількості сульфатованих глікозаміногліканів, знижується також вміст нейтральних глікозаміногліканів та глікопротеїнів їх ШІК-позитивні властивості помірно виражені.

Ключові слова: термічний опік шкіри, інфузійні розчини, щури-самці, гістохімічні дослідження.

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ДИНАМИКА ГИСТОХИМИЧЕСКИХ ИЗМЕНЕНИЙ В КОЖЕ КРЫС В ТЕЧЕНИЕ МЕСЯЦА ПОСЛЕ ОЖОГА ІІ-ІІІ СТЕПЕНИ НА ФОНЕ ВВЕДЕНИЯ ПЕРВЫЕ 7 СУТОК РАСТВОРА HAES-LX-5%

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Целью работы было изучить состояние эластичных, коллагеновых волокон, гликопротеинов и гликозаминогликанов в межклеточном веществе дермы крыс-самцов через 1, 3, 7, 14, 21 и 30 суток после ожога ІІ-ІІІ степени на фоне введения первые 7 суток раствора HAES-LX-5%.

Через 1 и 3 суток после термической травмы 2-3 степени в условиях применения раствора HAES-LX-5% реорганизация межклеточного вещества дермы проявляется меньшим повреждением волокнистых структур ее сосочкового и сетчатого слоев. Гистохимически аморфное вещество соединительной ткани богато на гликопротеины, содержание гликозаминогликанов еще невелико. 2. При введении HAES-LX-5% через 7 и 14 суток опыта при окраске по методу Ван Гизон-Вейгерта в соединительной ткани в краевых участках пораженной кожи наблюдается обновление коллагеновых волокон, они преимущественно расположены волнообразно, тонкими пучками. В поздние сроки опыта (через 21 и 30 суток) активный ход регенерации способствует значительной нормализации волокнистых структур дермы. В межклеточном веществе дермы нормализуется содержание соединений аморфного вещества, гистохимически установлено умеренное "Хейл"-положительное окрашивание, что свидетельствует об уменьшении количества сульфатированных гликозаминогликанов, снижается также содержание нейтральных гликозаминогликанов и гликопротеинов, их ШИК-положительные свойства умеренно выражены.

Ключевые слова: термический ожог кожи, инфузионные растворы, крысы-самцы, гистохимические исследования.

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