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EFFECTS OF THE DRUG "REOAMBRASOL" ON MORPHOLOGICAL CHANGES IN THE SKIN AND LIVER IN EXPERIMENTAL BURN SHOCK

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Abstract:

Burns continue to be one of the most socially significant aspects of the trauma problem. Despite the progress of combustiology and intensive care, the results of treatment of such patients often remain unsatisfactory. The pathogenetic substantiation and the development of a system of correction of organ dysfunction in patients with extensive deep burn injuries is an urgent task for practical medicine[10].

Keywords: Burn Shock, Morphological Methods Of Research, Infusion Blood Substitute "Reoambrasol", Rats, Skin

INTRODUCTION.

The liver is the main target organ in extensive burn injuries. Manifestations of cytolytic and cholestatic syndromes are observed in the first day of the disease[1]. In order to correct these conditions, a clear scheme of infusion intensive therapy is necessary. In burns it is difficult to achieve restoration of circulating blood volume only by infusion of plasma substitute solutions[9]. We need drugs that stabilize metabolic disorders and reduce the concentration of pro-inflammatory cytokines. Complex intensive therapy for burns should also include correction of cellular energy production without increasing oxygen transport. Substrate antihypoxants are used to reduce tissue oxygen demand, stabilize cell membranes, and reduce lipid peroxidation. Substrate antihypoxants include succinic acid preparations with detoxifying, antihypoxic, antioxidant and hepatoprotective effects [6]. The drugs modify cellular respiration, compensate metabolic acidosis, reducing the concentration of lactate, pyruvate and citrate, normalize the content of histamine and serotonin, improve microcirculation, without affecting systemic hemodynamics. All these effects are pathogenetically justified in the treatment of burn victims[7]. The study of various aspects of burn shock and the development of new methods of treatment, is still relevant, this is due to the high rates of lethal outcomes, which largely depend on such factors as the area of thermal burns, the depth of injury, as well as the time elapsed from the onset of thermal trauma to the provision of specialized care. An important condition for improving the results of treatment of patients with thermal trauma is the use of more effective methods of treatment, including infusion therapy. For this purpose, we used in the experiment a new infusion blood substitute "Reoambrasol", which has antioxidant, antihypoxic and detoxifying effects [5,8].

OBJECTIVE OF THE STUDY:

To study the effect of rheoambrasol on morphological changes in the skin and liver in experimental burn shock.

MORPHOLOGICAL METHODS OF INVESTIGATION:

The experiments were carried out on 80 male rats weighing 180-200g. The model of burn shock was reproduced by applying a 3x3 cm copper plate heated to 200°C to the back of the rat under ether anesthesia [3].

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The animals were divided into 4 groups: Group I (intact rats), Group II (control) - burn without treatment, Group III - burn shock followed by reopolyglucin infusion, Group IV - burn shock followed by reoambrasol administration.

At the end of the experiment, liver and skin fragments were taken for morphological examination. The pieces obtained from the skin and liver of experimental animals were first fixed in a neutral solution of 10% formalin for 48 h, then washed with running water for 4 h. Tissue fragments were dehydrated by immersion in a series of alcohols (70, 80, 96, 96, 100%) and chloroform, then paraffin wax was added. 5-6 µm sections were made from the paraffin blocks and stained with hematoxylin-eosin, the most commonly used method for staining histological sections [2].

Sections were dewaxed in chloroform, washed with distilled water, then a drop of haematoxylin solution was added to the slice surface and incubated for 3 minutes, then washed in running water for 10 minutes and stained for 0.2 to 3 minutes with Eosin. The preparation was then dehydrated in 70- and 96-degree alcohol, passed through carbol-xylene and xylene and covered with balsam. Result: cell nuclei were stained blue-black and cytoplasm was dark purple.

RESULTS AND DISCUSSION:

The results of the morphological study showed that in the skin and liver, no changes in the fragmentary structure were normally observed. Morphological studies of the skin in thermal burns revealed coagulation necrosis in all layers in animals (Fig. 1, 2) and various toxic intermediates (components of necrotic cells, partially oxidised intermediates, indole, scatole and other organic substances) pass through the blood to detoxify the liver

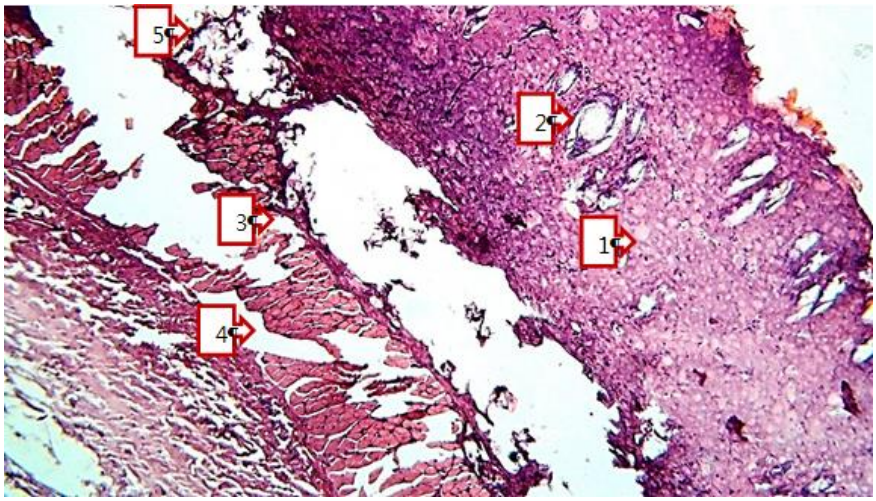

Figure 1: Morphological changes in the skin in burn shock

Multiple coagulation necrosis in the epidermis (1), desquamative necrosis of hair follicles (2), detachment of the dermis layer from other layers, coagulation of blood vessels (3), in the subcutaneous intermuscular spaces interstitial edema (enlargement) (4), many destructive and defragmented soft tissue components (5). Hematoxylin-eosin staining, 4x10

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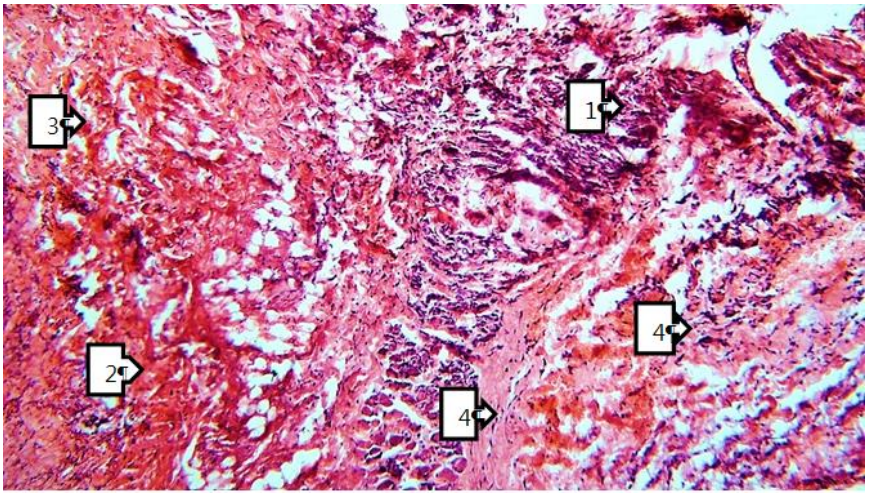

Figure 2: Morphological changes in the skin in burn shock.

The subcutaneous layer, coagulation necroses (1), haemorrhagic impregnated soft tissue (2), vascular atrophy and perivascular haemorrhages (3) are also evident. Destruction and defragmentation of fibrous structures and muscle components (4). Hematoxylin-eosin staining, 4x10.

From the pathophysiological point of view, the hyperdynamic type of response to thermal burns is accompanied by: increased body temperature, increased cellular oxygen and glucose requirements, increased glycogenolysis, lipolysis and proteolysis; the connective tissue structures in dermis are destroyed and undergo fibrinoid and coagulation necrosis (Ushakova T.A. 2008) [5, p. 1-50].

In hepatocytes it is manifested in varying degrees by morphological changes aimed at neutralization of intermediate products formed by thermal burn. Macroscopically the liver is slightly enlarged, capsule is slightly thickened, expressed changes were not detected by the naked eye, in vessels we observed fullness of different degree: dark brown in cross-section, dark foci with indistinct boundaries (signs of venous fullness) and alternating yellow foci, surface is even.

Microscopically the following changes were detected: the radial structure of the liver was partially altered, the perilobular veins were full of blood. Hepatocytes surrounding the triads were found to have hydropic dystrophy and fatty dystrophy in the form of large, medium and small droplets in the centrilobular hepatocytes. The cytoplasm of most hepatocytes develops hydrophobic staining and varying degrees of hydropic dystrophy (Fig. 3). At the same time, it was determined that the hepatocytes were solitary (shock cells). Foci of Kupffer cell proliferation were found in the sinusoids around the triads (Figure 4). At the same time, centrilobular and isolated perilobular monocellular foci of necrosis were detected

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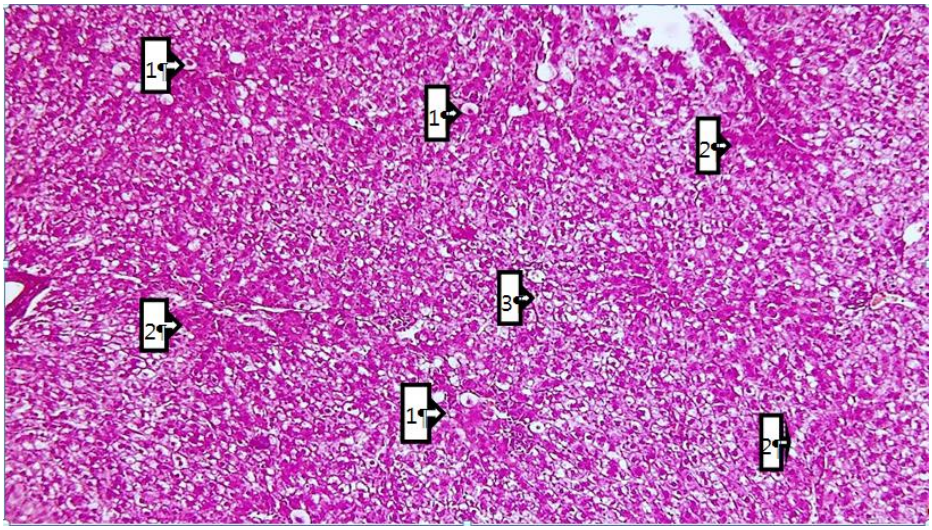

Figure 3: Morphological changes in the liver in burn shock.

The general background of the liver tissue shows hepatocytes with a large number of hydropic dystrophies (1), hepatocytes with dark cytoplasm around the triads are clearly shown (2), the membrane borders of hepatocytes with hydropic dystrophy are very well described (3). Hematoxylin Eosin dye, magnification 10x10

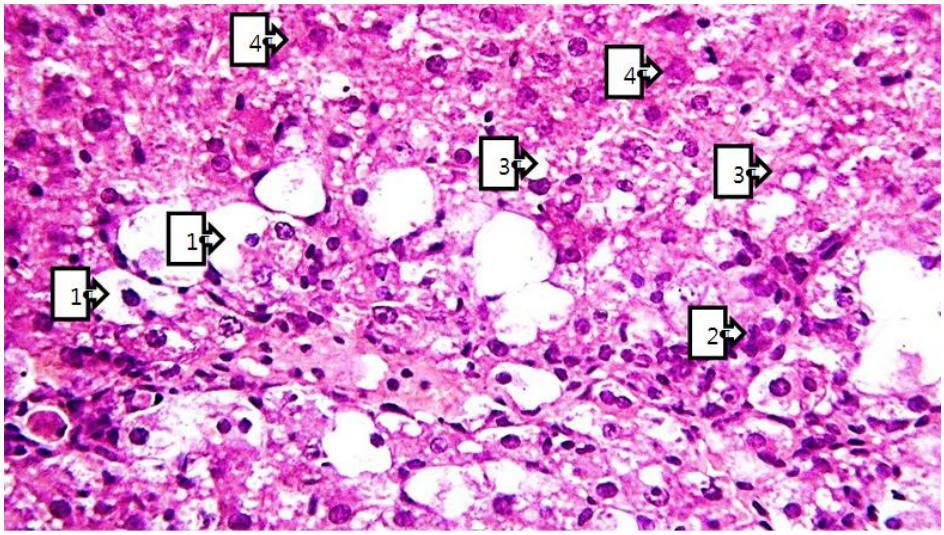

Figure 4: Morphological changes in the liver in burn shock

Liver tissue, balloon dystrophy in perilobular hepatocytes (1), with the nucleus in the centre in some and in others in the periphery. Kupffer cell proliferation around the triads is clearly described (2), with the cytoplasm of hepatocytes at the periphery of the fragment revealing fine-drop dystrophic foci (3). Hepatocyte cytoplasm is revealed as coarse-grained inclusions (4). Hematoxylin Eosin dye, magnification 10x40.

As a result, thermal burns simultaneously activate inflammatory mechanisms, vessels dilate, interstitial edema is detected in stromal elements, and serous inflammation develops in the affected areas. However, penetration of secondary bacterial infection into the area of injury leads to the appearance of serous purulent exudate. In response, deep hydropic and fatty dystrophic changes in the liver tissue lead to hepatocyte necrosis and fibrogenesis of the cells and liver fibrosis (Fig. 5). After the final formation of loose fibrous tissue, after a certain time, this tissue turns into coarse fibrous connective tissue. This eventually leads to the development of post-necrotic cirrhosis.

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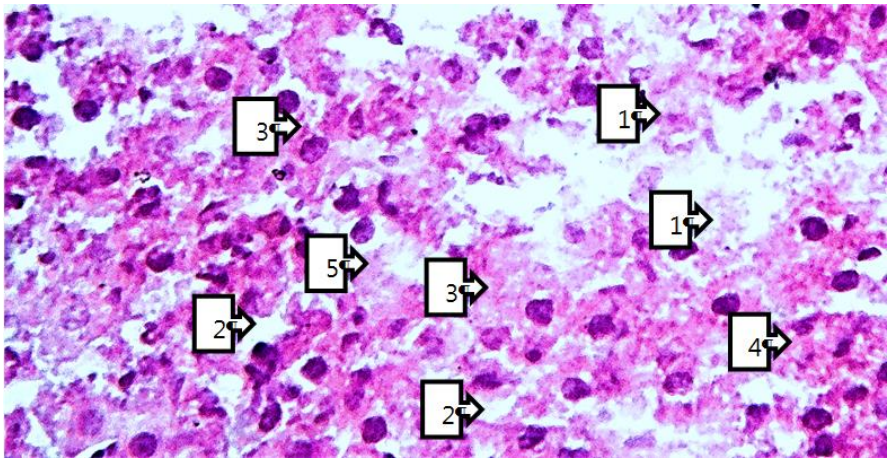

Figure 5: Morphological changes in the liver in burn shock

Liver tissue. Foci of necrosis of hepatocytes (1), different degrees of sinusoidal cavity overgrowth (2), basophilic stained granular structures (3) in hepatocyte cytoplasm are revealed in centrilobular area. Foci of fatty and hydropic dystrophy in which hepatocytes form (4). The cytoplasm of hepatocytes is stained differently (5). Hematoxylin-Eosin dye, size 10x60.

After intravenous administration of Reopolyglukin solution (group III) for 5 days morphological changes in skin and liver in burn shock were characterized by stimulation of detoxification properties of hepatocytes as well as general cytoprotective properties, increased scarring and regenerative activity of skin and its components. In particular, the solution of reopolyglucin injected into rats stimulated the formation of granulation tissue on burned areas of macroscopic skin. An increase in the number of active mitotic foci in the epidermal layers of the dermis was observed (indicating the cytoprotective effect of dextran in reopolyglucin) (Fig. 5). As a result of the hyperdynamic inflammation that developed in response to the damage, increased neutrophil infiltration was observed in the skin of group 2 rats (Fig. 6). This condition indicates a multiple enhancement of the inflammatory response. In the liver, Figure 6 shows a relative acceleration of metabolic processes in hepatocytes. The following changes were observed in the liver tissue under the influence of the drug "Reopolyglucin": the fragmentary radial structure was relatively restored, the sinusoidal cavities had the same width as in the liver structure of group II rats. Reduced foci of fatty and hydropic dystrophy formed in hepatocytes (Fig. 7), presence of foci of monocellular necrosis in the foci, slight signs of venous fulmination in the perilobular venous vessels. In group II rats, the dissection cavities shrank in size in response to changes in the liver (Figs 8, 9).

Thus, in response to thermal burns, an increased hyperdynamic inflammatory response, increased neutrophil infiltration in the inflammation focus, increased fibroblast proliferation, increased hepatocyte inflammation in damaged tissues, etc. developed under the effect of reopolyglucin

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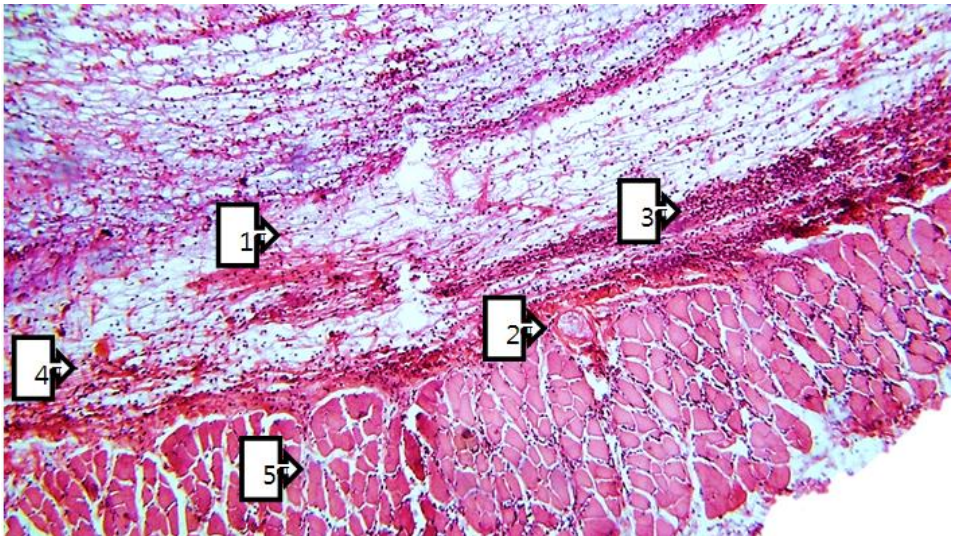

Figure 6: Morphological changes in the skin after treatment of burn shock with reopolyglucin

The dermis and hypodermis show foci of diffuse leucocytic infiltration after thermal burns (1). Blood vessels of derma and hypodermis (2) clearly show foci of perivascular leukocytic infiltration (3), loose fibrous connective tissue overgrowth in derma (4), interstitial edema between muscular bundles (5). Hematoxylin Eosin dye, magnification 10x10.

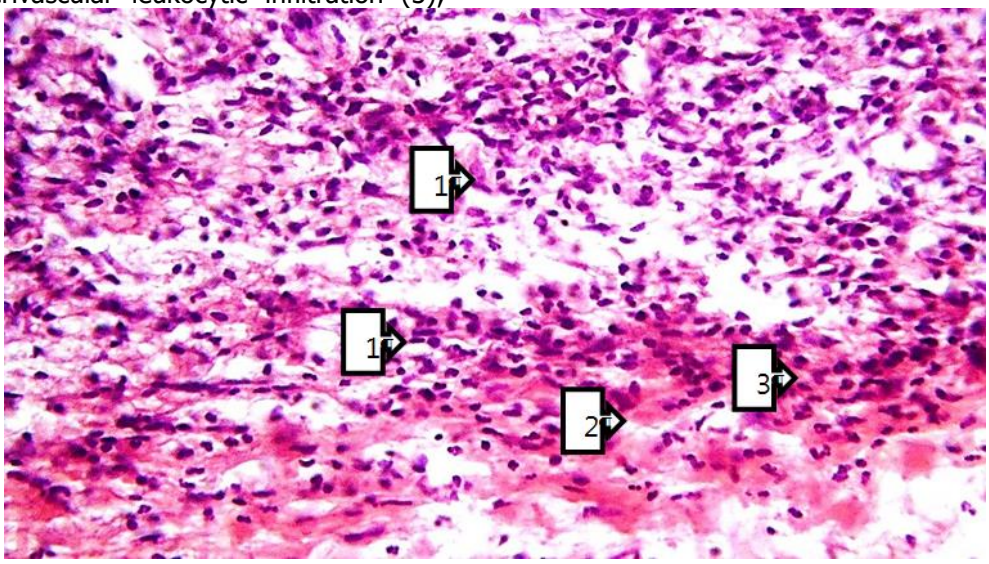

Figure 7: Morphological changes in the skin after treatment of burn shock with reopolyglucin

The dermis shows foci of infiltration of plasma cells and neutrophils around foci of necrosis (1). Foci of diapetal haemorrhage are seen in areas close to the basal membrane (2). Edema of varying degrees has developed in the stroma and interstitial spaces (3). Hematoxylin Eosin dye, magnification 10x40

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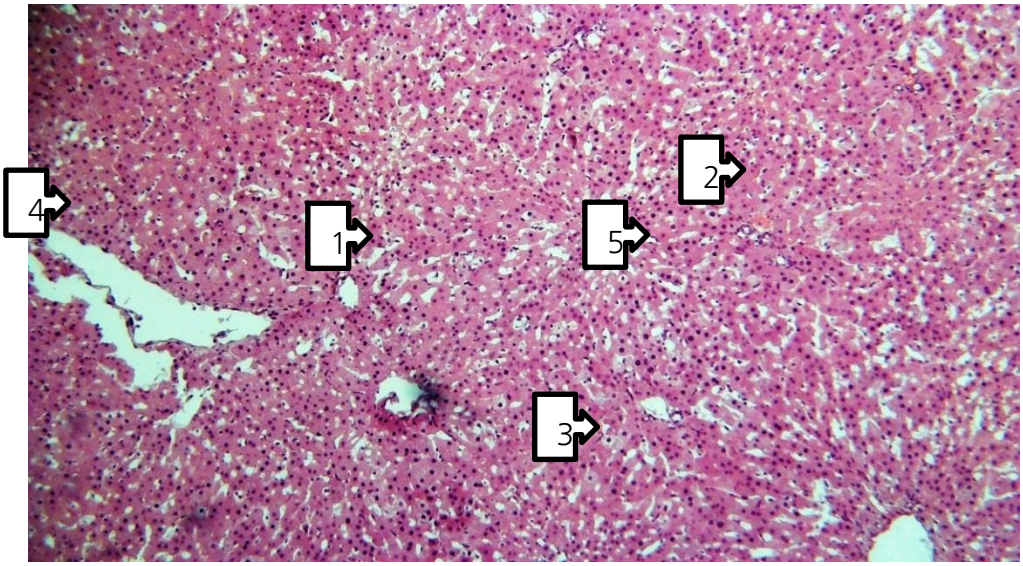

Figure 8. Morphological changes in the liver after treatment of burn shock with reopolyglucin

Liver tissue. The shape of the liver fragments (1), radial structure (2), sinusoidal spaces of different width (3), hepatocytes also of different size (4) remain in the general background. Perilobular venous vessels are fulvous (5). Hematoxylin Eosin dye, magnification 10x10.

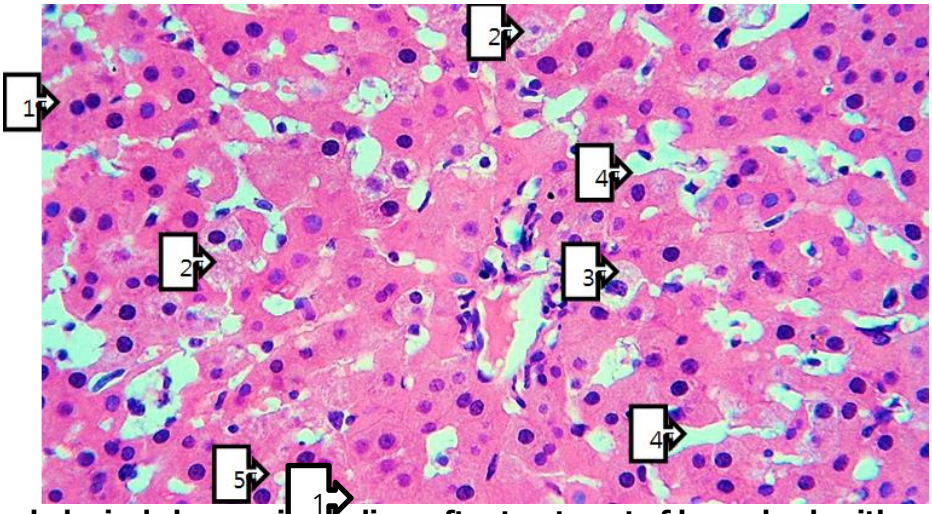

Figure 9. Morphological changes in the liver after treatment of burn shock with reopolyglucin.

The focus reveals normal mitotic foci around hepatocytes with hydropic dystrophy (1). Regeneration of hepatocytes undergoing hydropic dystrophy (2), formation of a homogenous pink structure in the cytoplasm (3), sinusoidal cavities of relatively different widths (4). Hematoxylin Eosin dye, magnification 10x40

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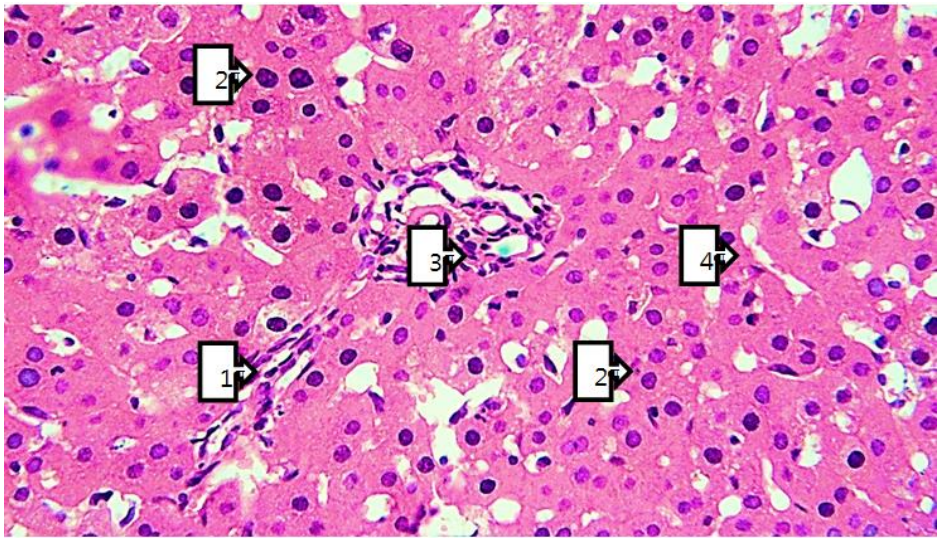

Figure 10. Morphological changes in the liver after treatment of burn shock with reopolyglucin

Fibroblast cells proliferating around the triads (1), anisochromia in the nucleus of most hepatocytes (2), focal hepatocytes with large blobs of fatty degeneration (3), sinusoidal cavities returning to their original state (4). Hematoxylin Eosin dye, magnification 10x40.

Studies of structural and morphological changes in the skin and liver after administration of reoambrasol for 5 days (group IV) showed that treatment restored the damage caused by burn shock. Thus in the skin: the process of reparative regeneration and scarring of the epidermis was completed by the process of replacement. The proliferation of vascular endothelium and the repair of parabasal cells in the deeply damaged basal layers of the dermis increased. This led to a release of cells with dystrophic changes in epithelial components from the dystrophic state and a decrease in hypoxia. As a result, restoration of the morphofunctional layers of the dermis and hypodermis was observed (Fig. 10, 11). After treatment with reoambrasol, the necrotic epidermis regenerates and recovers (Fig. 12)

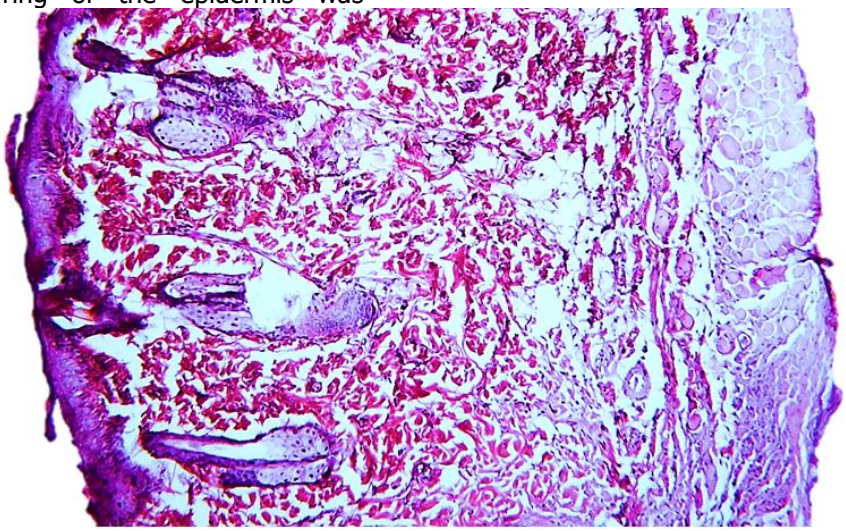

Figure 11. Morphological changes in the skin in burn shock after reoambrasol infusion.
Additional skin structures in the dermis are restored.
Hematoxylin-eosin staining, magnification 10x40.

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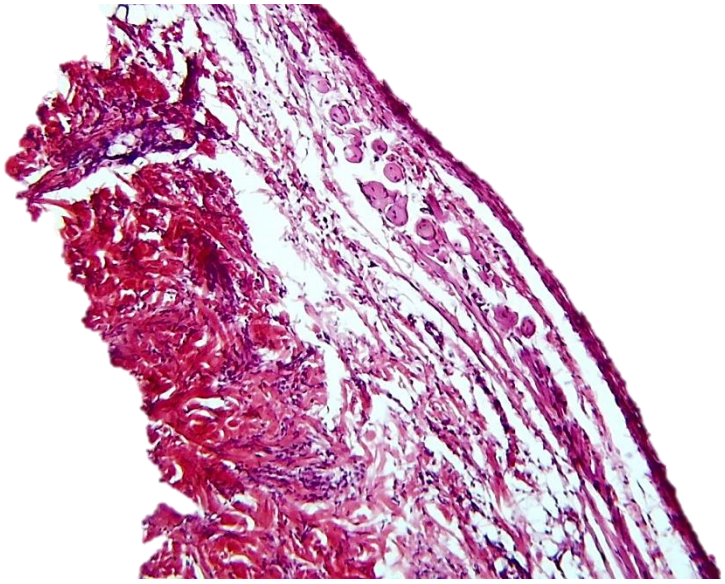

Figure 12. Morphological changes in the skin in burn shock after reoambrasol infusion

Regeneration of the epidermis of the skin surface, appearance of squamous epithelium. Hematoxylin-eosin staining, magnification 10x10.

A study of liver morphology after treatment with rheoambrasol revealed the following changes: the structure of the radial barrier of the liver returned to its normal structure. Hydropic dystrophy developed in the hepatocytes: light homogeneous structures in the cytoplasm became basophilic (a basophilic appearance is basically understood as normal staining of protein structures in the cytoplasm of healthy hepatocytes). In our study, Figures 12-13. show the regeneration of hepatocytes with edematous dystrophy under the influence of reoambrasol. In hepatocytes with large blobs of fatty dystrophy, regeneration to small blobs of dystrophy was observed and healthy hepatocytes are visible. Figure 13 shows the normal appearance of the sinusoidal cavities and the return of the Dissé cavities to relative normality after their enlargement. However, the increased mitotic activity of hepatocytes and the increased proliferative activity of Kupffer cells around the triads are more pronounced than the morphological changes in the liver of rats after infusion of reopolyglucin (group 3)

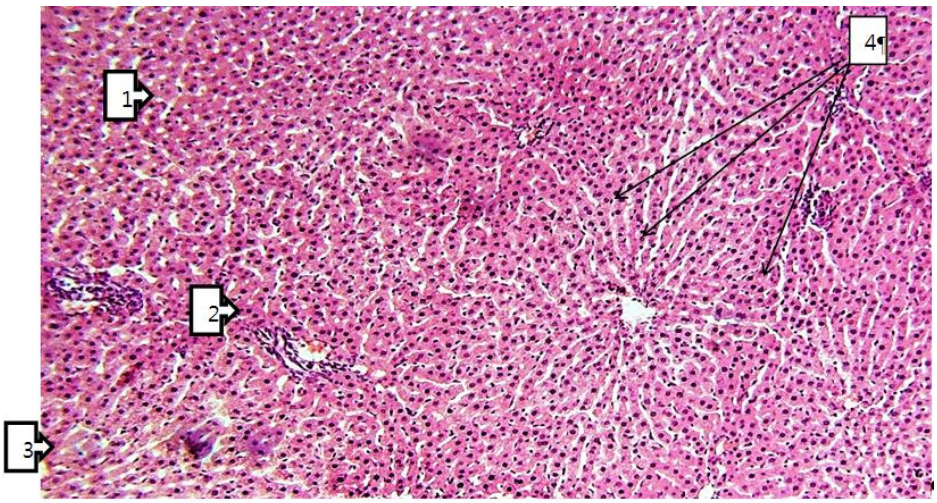

Figure 13.Morphological changes in the liver in burn shock after infusion of reoambrasol.

The cytoplasm of hepatocytes was stained homogeneously dark (1), pericellular borders were clearly delineated (2), foci of monocellular necrosis were identified in the focus (3), hepatocyte nuclei were stained dark. The sinusoidal spaces are almost equally wide (4), and fibroblast overgrowth around the

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triads is poorly formed (5). Most hepatocytes have a normal appearance (5). Hematoxylin Eosin dye, Magnification 10x10.

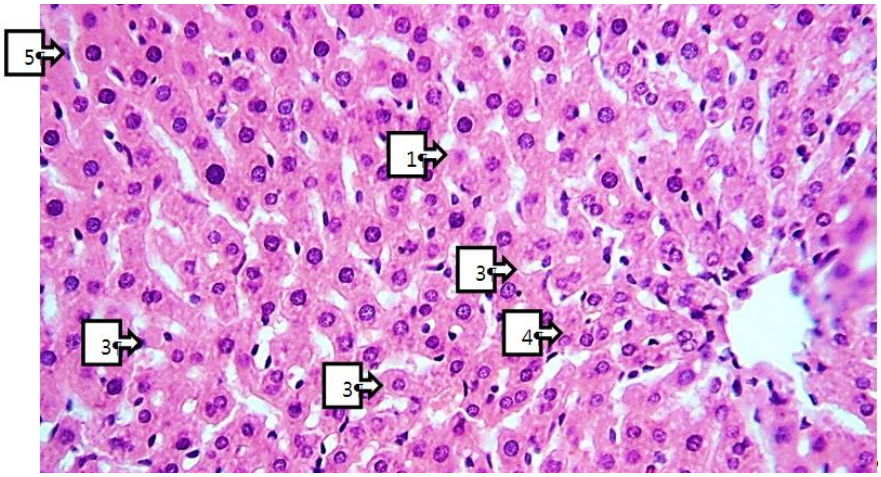

Figure 14. Morphological changes in the liver in burn shock after reoambrasol infusion.

Very small fatty dystrophic changes in the cytoplasm of hepatocytes on a large scale (1), formation of dark homogeneous protein structures in the cytoplasm of hepatocytes with hydropic dystrophy after Reoambrasol (2), sinusoidal cavities of equal width are revealed, Kupffer cells unchanged in places, centrilobular atrophically altered hepatocytes return to their former state(4), cytoplasm in perilobular hepatocytes homogenized (5) Hematoxylin Eosin dye, magnification 10x40.

Thus, the action of the blood substitute rheoambrasol in burn shock is much more effective than that of reopolyglucin. Blood substitute reoambrasol regenerates morphofunctional skin damage and restores the structure of liver tissue in burn shock, prevents hepatocyte necrosis and restores cell metabolism.

CONCLUSIONS:

1. burn shock led to the development of coagulation necrosis of the epidermis and dermis in the skin, severe hepatic dyscirculation, vacuolar and hyaline hepatocyte dystrophy, necrobiosis and eventually coagulation necrosis.
2.During treatment of shock by reopolyglucinum stabilization of dystrophy and necrobiosis processes in skin and liver tissue in comparison with control group, reparative regeneration of skin epidermis, increase of protein content in cytoplasm of hepatocytes, hyperchromasia of nuclei was observed.
3.When reoambrasol is used, skin regeneration and repair occur, destructive and necrobiotic changes of hepatocytes are sharply reduced, metabolic processes in cytoplasm are intensified, hyperchromasia of nucleus appears, histotopographic structure of cytoplasm of hepatocytes is close to normal.

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