Morphology of Reparative Regeneration in Organs and Tissues during Treatment with New Generation Sulfacrylate

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We studied morphogenesis of reparative processes in parenchymatous and hollow organs after surgery with the use of Sulfacrylate glue composition characterized by good adhesive and bactericidal properties. The glue rapidly and effectively arrested parenchymatous bleeding, reduced the volume of necrotic tissue, and promoted wound healing without suppuration. Cicatrix was completely formed 1 month after surgery and the glue completely resorbed at this term. The bioglue reliably connected and hermetically sealed sutures when used for gluing intestinal loops and for creation of intestinal anastomoses. The use of biological glue promoted the formation of elastic fibrous tissue not distorting the intestinal lumen.

Key Words: *partial resection of parenchymatous organs; intestinal anastomoses; reparative regeneration; cyanoacrylate biological glues; morphology*

The development and use of principally new forms of connection and hermetic sealing of sutures in zones of operative interventions are modern trends in the development of surgical technologies. Biological glue compositions creating reliable hemostasis are offered for solving these tasks. Among modern biological glues possessing a wide range of effects, chemical compositions based on α-cyanoacrylates possessing no carcinogenic and allergenic properties are often used in medical practice [1-3,6-10].

An important problem in introduction of new surgical technologies making use of bioglues is evaluation of the morphogenesis of reparative processes in sites of tissue injury and glue application. This approach allows evaluation of both positive and possible negative effects of the applied biohermetics.

Here we studied morphogenesis of reparative regeneration of parenchymatous and hollow organs after surgical interventions with the use of Sulfacrylate, a new glue composition.

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MATERIALS AND METHODS

An original third-generation glue composition Sulfacrylate was studied. This composition includes 2-cyanoacrylic acid ethyl ether (70-85 g), 2-methylacrylic acid 1,1-dioxotetrahydro-1λ-thiophene-3-ylic ether (7.5-15.0 g), and acrylic acid butyl ester (5-15 g) [4,5].

Experiments were carried out on 142 animals (125 rats, 12 mongrel cats, and 5 Chinchilla rabbits). The interventions were carried out in an experimental operating room under aseptic and antiseptic conditions. Tissue fragments were resected from the liver, spleen, and kidney; interventions on the intestine included gluing of the serosa and strengthening of intestinal anastomoses. The animal status was daily controlled during the postoperative period. The effect of bioglue on tissues of parenchymatous organs and intestine was studied during repeated surgeries at various terms (up to 3 months). The competence of intestinal anastomoses was studied by the pneumopressure method.

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Control groups consisted of animals subjected to similar surgery without glue application.

Pathomorphological study of tissue fragments dissected in the operation zone was carried out. The rats were decapitated under ether narcosis, cats and rabbits under hexenal or thiopental narcosis combined with short-acting myorelaxants. Tissue fragments were fixed in 10% neutral formalin. Samples for microscopic examination were prepared by routine methods, the sections were stained with hematoxylin and eosin and by the method of Van Gieson.

RESULTS

Application of the glue onto the wound surface of the liver, spleen, and kidneys after their partial resection resulted in the formation of a polymeric film ensuring hemostasis. Three hours after the intervention migration of leukocytes to the wound surface was observed and small focal hemorrhages were seen in the parenchyma.

After 6 h necrotic zones of different size formed at sites of organ resection and glue application. In the liver the thickness of the necrotic area corresponded to approximately half of the hepatic lobe diameter. In the spleen the width of necrotic zone approximated the diameter of the follicle. In the liver the necrotic zone contained small islets of viable hepatocytes (though with degenerative changes). We observed small leukocytic infiltrations in liver areas adjacent to the necrotic zone. In the spleen solitary lymphocytes and preserved follicles were seen in the necrotic zone. Areas adjacent to the necrotic zone were also infiltrated with leukocytes.

In the kidney hydropic degeneration of epitheliocytes in the excretory tubules near the wound surface was observed 6 h postoperation. Slight leukocytic infiltration was observed in the zone of glue application. Congestion was seen in the glomeruli and excretory tubules in sites distal from the wound.

Morphological changes in the parenchymatous organs 12 h after their partial resection were caused by surgical trauma and local toxic effect of the glue, which led to the development of aseptic inflammation (necrobiosis and cell necrosis, hemorrhage). The necrotic zone in the liver (Fig. 1, *a*) and spleen (Fig. 2, *a*) was more clearly seen at this term. In the liver leukocytic infiltration formed at the interface between the wound surface and normal tissue and in the portal tracts, but necrosis did not involve deeper layers of the parenchyma.

Reparative regeneration of the kidney at the site of the operation wound treated with the glue was associated with minimum damage: small focal necroses of epitheliocytes in the excretory tubules were separated from the adjacent tissue (Fig. 2, *b*). The glomeruli and excretory tubular epithelium remained intact in sites distal from the wound.

In control animals operated without glue application using traditional methods of hemostasis (catgut suture) extensive necrotic zones with abundant polymorphonuclear infiltration (Fig. 1, *b*) and hemorrhagic foci (Fig. 2, *c*) were seen in organ specimens at the same terms of the experiment.

Twenty-four hours after resection the necrotic zone was clearly separated from viable tissue in the liver and spleen. Pronounced inflammatory infiltration was seen in liver areas adjacent to the glue layer (Fig. 1, *c*). Numerous destroyed leukocytes and small solitary islets of viable tissue were seen in this zone. Moderate lymphohistiocytic infiltration with solitary granulocytes was seen in the portal tracts. In the spleen scanty polymorphonuclear infiltration was observed in areas adjacent to the glue layer and hemorrhages were seen at the interface with normal tissue. The number of cells in the follicles decreased in the necrotic zones, hyperplastic reactions were seen outside the necrotic zone. A necrotic zone, though a narrower one, appeared in the zone of glue contact with the capsule.

One week after surgery a narrow necrotic zone separated from viable tissues was seen in the resected liver lobe under the glue; initial signs of necrotic mass organization manifesting in intensification of fibroplastic reactions at the necrosis borderline were observed. Moderate polymorphonuclear inflammatory infiltration of liver areas adjacent to the glue was noted. Similar changes were seen in the spleen. Abundant polymorphonuclear infiltration appeared in splenic areas adjacent to the wound surface covered with the glue. Many destroyed leukocytes were seen in the infiltrate. The follicles were formed mainly by young lymphocytes. Partial resorption of the glue was observed at resected surfaces of the liver and spleen at this term.

One month after partial resection with subsequent application of Sulfacrylate necrotic zones with forming cicatrix were seen in the liver (Fig. 1, *d*), spleen and kidney (Fig. 2, *d*). This was paralleled by complete resorption of the glue. Comparison of morphological changes in the tissues in experimental and control group showed more intensive inflammatory reaction in the control. Cell necrosis and massive hemorrhages in the wound in control preparations were caused by tissue damage not only during resection, but also during suturing. Moreover, in controls the wounds healed with purulent inflammation (with microabscesses).

Similar results were observed after the fixation of the intestinal loops with Sulfacrylate. The glue reliably fixed intestinal loops to each other and the adhesion

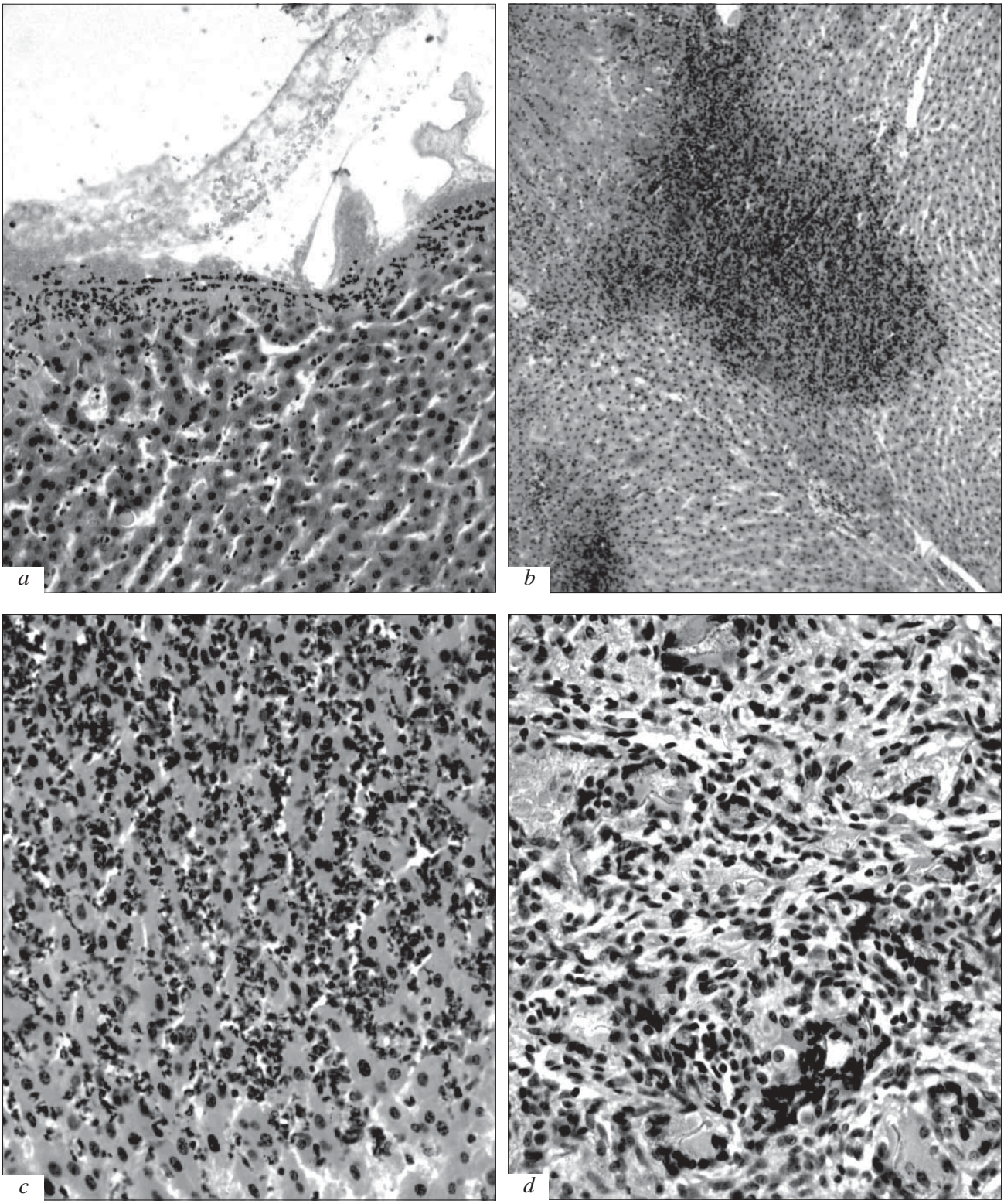

Fig. 1. Morphological changes in the rat liver after partial resection. Here and in Fig. 2: hematoxylin and eosin staining. *a*) liver wound treated with Sulfacrylate 12 h after resection, ×200; *b*) formation of a microabscess near the wound surface not treated with the glue 24 h postoperation, ×100; *c*) leukocytic infiltration of the zone adjacent to the wound surface treated with the glue 24 h postoperation, ×200; *d*) formation of a cicatrix in the liver resection zone 1 month after the intervention with glue application, ×400.

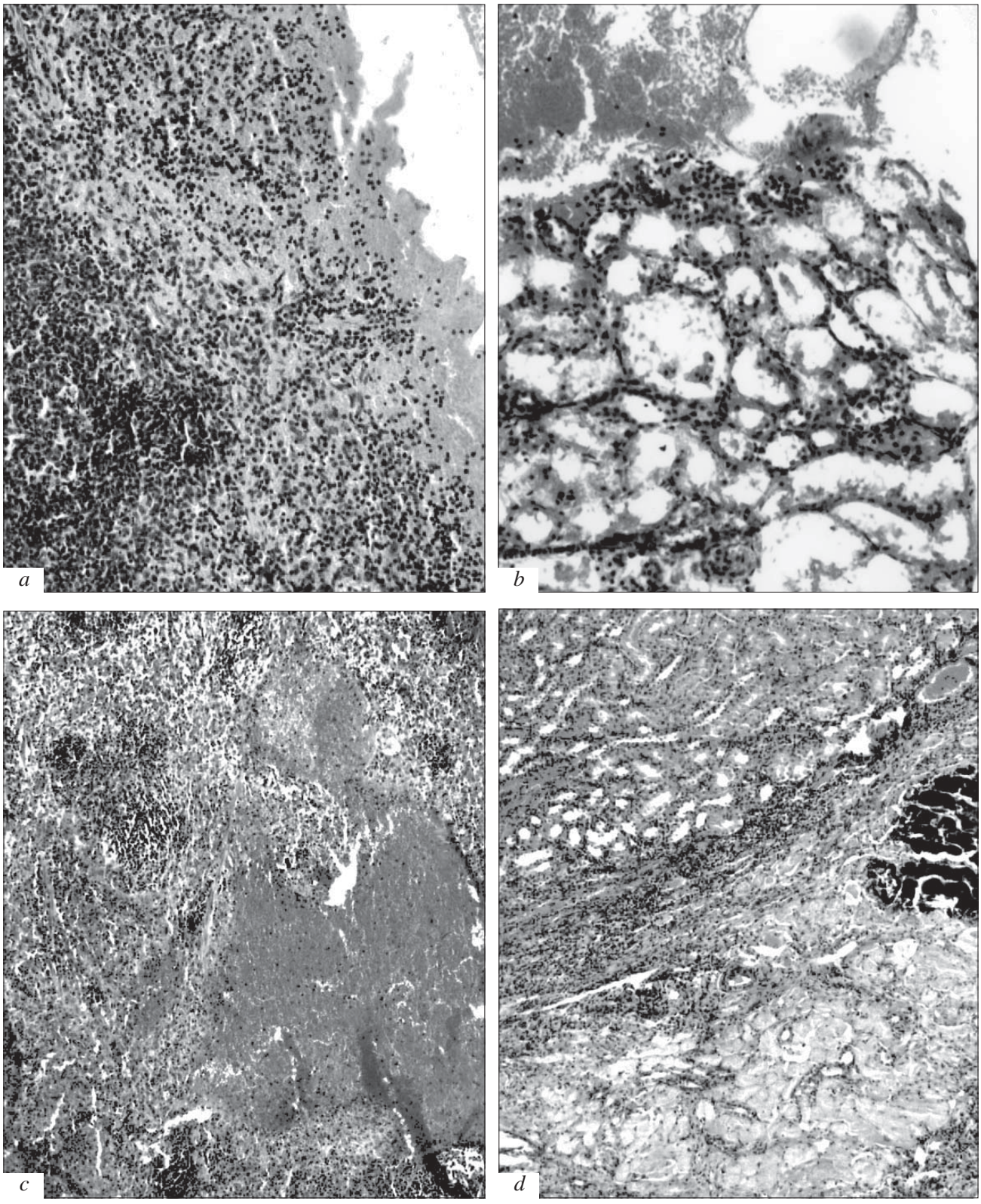

Fig. 2. Morphological changes in the rat spleen after partial resection. *a*) the spleen 12 h after resection and treatment with Sulfacrylate, ×200; *b*) renal excretory tubules necrosis in the resection zone 12 h after operation with the glue application, ×200; *c*) massive hemorrhages in the spleen 12 h after resection without the glue application, ×100; *d*) formation of a cicatrix in the kidney necrosis zone 1 month after partial resection with glue application, ×100.

well resisted aggressive media during histological processing. On the other hand, edema of the serosa treated with the glue was observed at the initial stage of the experiment. Plasma impregnation and loosening of the mesothelium, appearance of individual lymphocytes and plasma cells were observed.

Six hours after the start of the experiment fibrin fibers oriented in the plane corresponding to the longitudinal axis of the intestine appeared in the thickened serous membrane of almost all examined fragments of the intestine. Newly formed adhesions spreading from one intestinal wall to the other or connections consisting of fine collagen fibers and fibrin were seen in some visual fields.

Connective-tissue fibers appeared in the zone of glue application 1 week after the start of the experiment. Granulation tissue with numerous macrophages, phagocytizing the glue, developed at sites where the intestinal loops were glued. Granulation tissue was enveloped in a well formed capsule. After 1 month the wound was covered with fibrous tissue and the glue was completely resorbed. Three months after the start of the experiment the intestinal function was normal in all cases, no macroscopic changes (changes in the diameter, wall thickness, or structure of the mucosa) were seen.

Morphological study of intestinal anastomoses was carried out. At early terms postoperation (12 h - 3 days) they were characterized by tight contact of the edges of the dissected intestinal walls. No pronounced alterations in the muscular layer and serosa were observed. Reliable hermetic closure of the intestinal suture was attained, there were no manifestations of peritonitis or adhesions with the adjacent organs.

Six days postoperation the intestinal wound edges were still tightly closeed, but hemodynamic disorders developed in the muscular layer (edema, venous plethora, and lymphostasis). After 1 month no signs of damage to the intestinal wall were seen, focal lymphohistiocytic infiltration persisted, mainly near the suture. Granulation tissue developed in the subperitoneal layer between retained structural components of the intestinal wall.

Histological study of intestinal anastomoses in control animals showed pronounced purulent inflammation (purulent anastomositis). Measurement of pneumopressure resistance of anastomoses treated and not treated with the glue showed advantages of the gluing technology over the traditional method (control).

Hence, analysis of reparation of parenchymatous organs after their partial resection and Sulfacrylate application showed good adhesive characteristics of the glue, which was fixed on the wound surface as a polymeric film, providing hemostasis and protecting the wound. The formation of coagulation necrosis zone was caused by local toxic effect of Sulfacrylate. The developing inflammatory reaction was aseptic. Cicatrization process was completed 1 month after the intervention, the glue was completely resorbed at this term. The severity of the inflammatory reaction after glue application varied, depending on the organ and severity of injury (most severe in the liver, least so in the kidney).

Application of the glue onto intact serous integument and suture line in creation of an end-to-end intestinal anastomoses confirmed the positive characteristics of the glue, which did not prevent tissue regeneration due to rapid separation of the coagulation necrosis zone and aseptic nature of the inflammation. The use of the glue promotes the formation of elastic fibrous tissue not constricting the intestinal lumen.

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