Praca oryginalna *Original paper*

Effect of early excision of a burn wound on the activity of selected enzymes in pig serum

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Summary

Thermal stimuli acting on the skin can cause burn wounds. Clinical and experimental studies suggest a beneficial effect of the necrectomy of post burn necrosis in the hyperacute phase and in the acute phase (during the initial period of burn disease). Heat stroke causes hypovolemic shock and influences the energetic status of the liver, which is the main organ responsible for energy production and energy storage. Determination of the activity of indicator enzymes in serum makes it possible to assess the clinical condition of animals. A total of 25 White Large Landrace pigs of both sexes weighing 50 kg (± 2 kg) were used for the study. Burns were made with a software-controlled heating plate on a precisely defined contact surface of 1% of body surface. This resulted in a burn rate of 30% (± 2%) of the body surface with a lesion range between grades III and IIb. Animals from each experimental group were subjected to an infraclavicular necrectomy. Blood was collected and analyzed for AlAT, AspAT, LDH, and CK activities. In all groups, a statistically significant ($p < 0.05$) increase in AlAT, AspAT, LDH, and CK activities was observed already 12 hours after the burn, and there was a very high correlation between the activities of parenchymal enzymes AlAT and AspAT. The study has shown an association between the moment of performing burn wound necrectomy and the activity of AlAT, AspAT, LDH, and CK.

Keywords: burn, necrectomy, hypovolemia, burn wound

High temperature disrupts the homeostasis of living organisms and leads to numerous pathologies in tissues and organs. Grade IIb and III burns cause local inflammatory changes and systemic reactions, whose initial phase is the systemic inflammatory response syndrome (SIRS). A burn exceeding 10% of the skin surface disturbs the intracorporeal balance and frequently causes the multiple organ failure syndrome (MODS) and hypovolaemic shock (5, 9). The burn wound being converted is a source of high concentrations of biologically active inflammatory mediators, including free oxygen radicals (ROS) and cytokines, which cause systemic reactions (8, 16). The severity and duration of these reactions depend on the area and depth of thermal injury and the area of contact between healthy and dead tissues. Stimulated phagocytes are also a source of ROS after burns. Rapidly increasing concentrations of proinflammatory substances damage biological structures and cause peroxidation of lipids, proteins, sugars, and nucleic acids. The reactions in burnt tissue take the form of an uncontrolled cascade. The generation and persistence of elevated concentrations of proinflammatory mediators after a burn induces behavioral responses, negative energy balance in the body, weight loss, and impairment of the immune system (5, 23). A severe burn causes damage not only to tissue at the site directly exposed to the thermal agent, but also to distant organs (14). This is manifested, among others, by changes in the activity of indicator enzymes and other biochemical blood parameters that determine the state of intracorporeal homeostasis (including those that describe energy, protein, and mineral metabolism, the functional state of the liver and the muscle system). Clinical and experimental studies indicate a significant effect of wound excision/burn necrosis performed in the hyperacute phase and in the acute phase of burn disease on the maintenance of body homeostasis (3, 11). The aim of this study was to evaluate the effect of necrectomy of a burn wound on selected biochemical parameters that describe the state of the organism after the burn and to determine the optimal time for excision of the burn wound.

Material and methods

A total of 25 healthy White Large Landrace pigs of both sexes weighing 50 kg (± 2 kg) were used in this study. The animals were provided with a day/night lighting system and received daily doses of balanced swine food and water *ad libitum*. The acclimation period for all pigs lasted seven days. The pigs were starved for 24 hours before the experiment. They were divided into 5 groups of 5 individuals each (Tab. 1). The consent for the research was granted by the 1st Local Ethics Committee for Animal Experiments of the Jagiellonian University in Kraków (resolution no 133/2014).

The anesthetic protocol followed procedures described in previous reports (1). Burns were performed with a software-controlled hotplate in a precisely defined contact area on a flank of the body equal to 1% of the body surface area in a 50 kg pig (2). The hotplate weighing 2.5 kg rested on the animal's body for 10 seconds without any additional pressure being applied (Polish patent no. 213590). The temperature of the hotplate was set at 200°C to obtain a burn rate of 30% (± 2%) of the body surface with a lesion range between grades III and IIb. The body surface area (BSA) burnt in each pig was precisely determined by the mathematical formula BSA (cm^2) = 734 BW 0.656 kg (25). Necrectomy (N) was performed as excision of necrosis down to the muscle fascia after 12, 24, or 60 hours. Blood samples were collected from the jugular vein before the start of the study (time 0) and then at hours 12, 24, 36, 48, 60, 72, 84, and 96 after the burn. Serum samples collected were evaluated for alanine aminotransferase (AlAT), aspartate aminotransferase (AspAT), creatine kinase (CK), and lactate dehydrogenase (LDH) activities. An automatic biochemical analyser BS-130 Shenzhen Mindray Bio-Medical Electronics Co., Ltd. was used in the study.

Tab. 1. Experimental groups

Group of animals	Type of injury
I	burnt without necrectomy
II	burnt with necrectomy at hour 12 after injury
III	burnt with necrectomy at hour 24 after injury
IV	burnt with necrectomy at hour 60 after injury
Control	not burnt, no necrectomy

Results and discussion

Changes in serum enzyme activities during the study are presented in Figures 1, 2, 3, and 4. The values of correlations between parameters are presented in Figure 5 and Table 6. In all groups, a statistically significant ($p < 0.05$) increase in the activity of all enzymes examined was observed already at hour 12 after the burn. In group I, AlAT activity increased until hour 96 ($p < 0.05$), ($r = 0.705$). In group II, AlAT activity decreased at hour 12 after necrectomy. It decreased in group III at hour 36 (i.e. 12 hours after necrectomy) and decreased to 150.8 U/L in group IV at hour 84 (i.e. 24 hours after necrectomy) ($p < 0.05$).

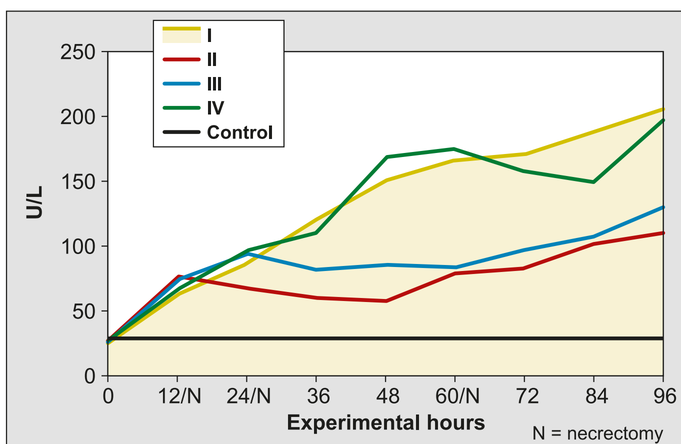

Fig. 1. Dynamics of changes in serum AlAT activity

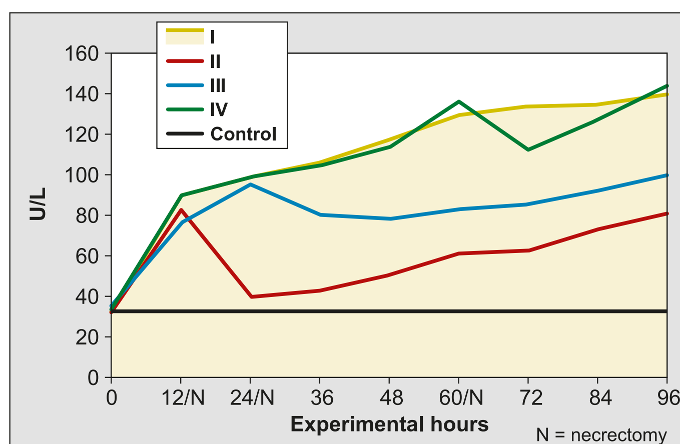

Fig. 2. Dynamics of changes in serum AspAT activity

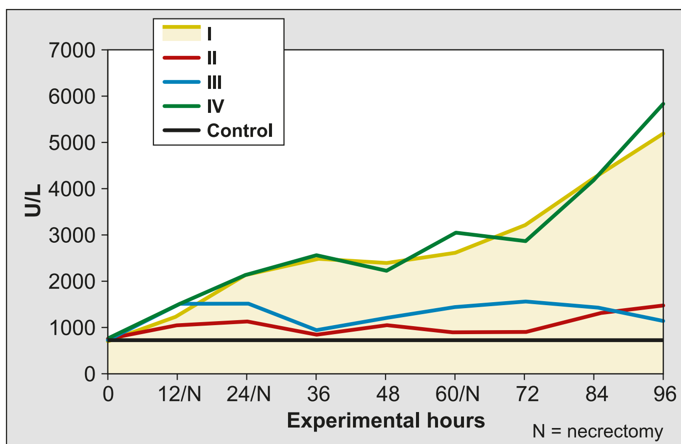

Fig. 3. Dynamics of changes in serum LDH activity

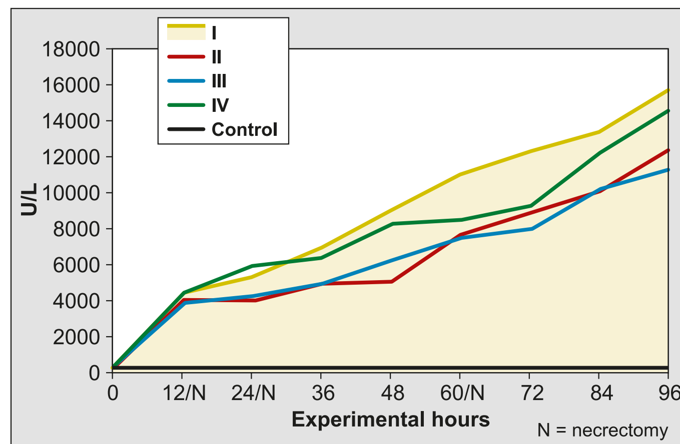

Fig. 4. Dynamics of changes in serum CK activity

In group I, AspAT activity increased until hour 96 ($p < 0.05$) ($r = 0.630$). In group II, AspAT activity decreased at hour 24 (i.e. 12 hours after necrectomy). In group III, it decreased at hour 36 (i.e. 12 hours after necrectomy). In group IV, it decreased ($p < 0.05$) at hour 72 (i.e. 12 hours after necrectomy).

There was a very high correlation between changes in AlAT and AspAT activities in all study groups, which

Tab. 2. Serum AlAT values

Group of animals	Average ± SD								
I	25.30 ± 0.89	62.70* ± 1.43	86.7* ± 2.93	120.6* ± 2.93	150.6 ± 2.81	166.7 ± 2.81	170.9 ± 0.90	188.2* ± 1.86	205.7* ± 1.88
II	28.20 ± 2.87	76.70* ± 1.77	66.9* ± 1.95	59.9* ± 2.32	56.9* ± 2.42	78.9* ± 3.32	82.9* ± 2.76	101.4* ± 2.68	110.6* ± 1.84
III	25.16 ± 2.21	73.80* ± 2.69	94.9* ± 3.28	81.4* ± 3.24	86.3* ± 3.83	83.2 ± 1.73	96.9* ± 2.79	107.2* ± 3.10	130.4* ± 2.96
IV	27.92 ± 2.55	66.74* ± 2.61	97.4* ± 1.89	110.2* ± 2.14	169.3* ± 2.13	174.7* ± 2.13	156.8* ± 2.09	150.0* ± 2.53	198.3 ± 2.77
Control	29.20 ± 0.55	29.30 ± 2.74	28.6* ± 1.44	29.5* ± 1.6	29.8 ± 2.33	29.9 ± 1.42	29.5* ± 2.06	30.6 ± 1.85	29.8 ± 2.57
Experimental hour	0	12	24	36	48	60	72	84	96

Explanation: * $p < 0.05$

Tab. 3. Serum AspAT values

Group of animals	Average ± SD								
I	32.2 ± 2.24	88.8* ± 4.18	98.5* ± 8.78	105.6* ± 3.82	116.8* ± 3.20	128.8* ± 2.91	132.9* ± 2.99	133.8* ± 1.61	138.9 ± 2.54
II	29.8 ± 3.00	82.6* ± 3.61	39.2 ± 3.78	42.7* ± 3.01	50.3* ± 3.20	60.5* ± 3.30	62.5* ± 2.85	72.8* ± 2.39	80.7* ± 1.79
III	35.5 ± 3.36	75.7* ± 3.61	94.5* ± 3.45	79.7* ± 2.21	77.9* ± 2.57	82.5* ± 2.79	85.2* ± 3.85	91.5* ± 3.38	98.9* ± 3.25
IV	32.9 ± 3.41	89.3* ± 7.03	98.8* ± 5.99	103.9* ± 6.62	112.8 ± 6.62	135.8* ± 6.05	111.8* ± 5.05	126.9* ± 4.96	142.8* ± 4.32
Control	31.2 ± 3.04	32.3* ± 3.73	32.8 ± 3.88	32.8* ± 4.22	32.8 ± 3.65	32.0 ± 2.87	32.4 ± 2.67	32.5 ± 2.54	32.4 ± 3.64
Experimental hour	0	12	24	36	48	60	72	84	96

Explanation: * $p < 0.05$

Tab. 4. Serum LDH values

Group of animals	Average ± SD								
I	790.9 ± 51.05	1280.3* ± 86.85	2161.0* ± 88.37	2475.6* ± 99.54	2389.1* ± 100.12	2628.2* ± 97.24	3234.9* ± 86.25	4273.1* ± 74.64	5200.4* ± 97.29
II	720.6 ± 97.29	1051.5* ± 89.11	1145.8* ± 76.54	864.9 ± 76.54	1049.6 ± 85.8	907.1* ± 82.16	914.3* ± 89.36	1289.2* ± 83.88	1489.9 ± 83.88
III	800.9 ± 93.43	1482.3* ± 78.31	1532.2* ± 74.35	956.0* ± 49.51	1226.8* ± 87.51	1472.6* ± 94.31	1565.5* ± 81.46	1448.3* ± 88.28	1140.3* ± 97.69
IV	789.6 ± 123.8	1507.0* ± 109.65	2178.3* ± 168.94	2575.3* ± 118.43	2211.8* ± 154.59	3053.9* ± 132.58	2862.9 ± 113.86	4194.5 ± 124.38	5865.8 ± 98.07
Control	720.7 ± 93.76	730.9 ± 65.90	722.2 ± 54.90	740.6* ± 45.90	720.7 ± 34.90	766.9* ± 43.60	755.5 ± 33.30	720.5 ± 44.70	720.7 ± 38.80
Experimental hour	0	12	24	36	48	60	72	84	96

Explanation: * $p < 0.05$

Tab. 5. Serum CK values

Group of animals	Average ± SD								
I	232.6 ± 86.6	4427.7* ± 215.9	5309.0* ± 275.80	6888.9* ± 245.70	8983.6* ± 324.30	10966.4* ± 257.36	12267.9* ± 257.36	13345.8* ± 299.24	15743.4* ± 245.75
II	217.9 ± 92.3	3968.7* ± 127.8	4009.4* ± 145.03	4896.5* ± 151.01	4987.8* ± 215.78	7674.5* ± 297.58	8888.6* ± 189.97	10097.8* ± 140.77	12344.6 ± 110.07
III	243.9 ± 88.9	3876.9* ± 289.6	4209.0* ± 310.60	4879.2* ± 280.62	6184.0* ± 221.10	7471.9* ± 214.23	8002.9* ± 231.90	10121.2 ± 134.92	11256.9 ± 115.60
IV	254.6 ± 94.8	4329.9* ± 287.3	5893.7* ± 277.46	6336.94* ± 283.69	8208.6 ± 228.52	8424.0* ± 218.45	9209.04 ± 154.83	12180.1 ± 195.82	14568.9* ± 204.20
Control	224.5 ± 98.9	232.4 ± 112.8	235.6 ± 116.90	245.1* ± 121.20	235.5 ± 89.60	243.4 ± 76.40	235.5 ± 78.40	233.3 ± 88.80	234.4 ± 79.60
Experimental hour	0	12	24	36	48	60	72	84	96

Explanation: * $p < 0.05$

indicates the influence of inflammatory mediators on the function of organs, including the liver, after the burn (Tab. 6). LDH activity showed a statistically significant increase ($p < 0.05$) in all experimental groups, starting at hour 12 after the burn. In group II, there was a slight decrease in LDH activity after necrocremation, which persisted from hour 36 to 48 of the study. In group III, after the necrectomy was performed, a decrease in LDH activity was noted at hour 36. This decrease persisted until hour 60 of the study ($p > 0.05$). After this time, LDH activity increased, reaching a maximum value of 1140.3 U/L at hour 96. In group IV, a decrease in LDH activity was noted at hour 72 after necrectomy.

A significant increase in CK activity ($p < 0.05$) was observed in all experimental groups, as early as hour 12 after the burn. After necrectomy, there was no decrease in CK activity below that noted at hour 12 of the experiment in any of the study periods. In groups in which necrectomy was performed, the activity at hour 96 of the study was significantly lower than it was in group I. There was also a high positive correlation between CK and LDH in experimental groups II, III, and IV (r = 0.723-0.952).

As an important supplement to the basic clinical examination, the examination of the activity of indicator enzymes (AlAT, AspAT, LDH, CK) in serum is helpful for the physician in making therapeutic decisions in cases where changes in cell membrane permeability or tissue traumatisation (4, 19) occurred. Under physiological conditions, the serum activity of indicator enzymes of cytoplasmic (LDH, AlAT, CK), mitochondrial (AspAT), and reticular (esterases, reductases, Glu-6-P phosphatase) origin is low. Increased activity appears after organ damage and depends on the degree of damage, which corresponds to the amount of damaged cells. Our study has shown that subfascial necrectomy in 30% p.c. burns of grades III and IIb performed 12 hours after the burns is more effective in reducing AlAT and AspAT activities than the same procedure performed 24 or 60 hours after the burns. Assuming that the liver is the main source of AlAT and AspAT, it can be concluded that the reduction of increase in their activity after necrectomy is associated with the reduction of paracrine action of inflammatory mediators formed in the burn wound as a result of its conversion. The activity of these enzymes is influenced mainly by the excessive generation of ROS, including iNOS, whose source is tissue necrosis following the burn (3, 8, 10). Higher AlAT activity compared to AspAT activity may be due to hepatocyte membrane damage caused by oxidative stress factors. Rhabdomyolysis following a massive trauma, including burns, is indicated as a cause of systemic inflammatory response (SIRS) and organ failure. A strong increase in myoglobin concentration and released Fe ions stimulate free radical reactions (20, 21). This entails an increase in the activity of indicator enzymes (20, 21). The results of our study show that high parenchymal enzyme activities were influenced by decompartmentation products derived from postburn necrosis rather than by effects associated with burn wound excision. LDH is classified as a cytosolic enzyme, and its concentration in the cell is several times as high as it is in serum. Mechanical damage to the cytoplasmic membrane of the cell (e.g. by chemicals or physical agents) causes serum LDH activity to increase (7, 15). Increased serum LDH activity is observed in

Tab. 6. Value of the correlation coefficient r between the parameters

		ALaT				AspaT				CK			
		I	II	III	IV	I	II	III	IV	I	II	III	IV
LDH	I	0.912				0.828				0.943			
	II		0.817				0.651				0.723		
	III			0.482				0.627				0.803	
	IV				0.803				0.822				0.952
CK	I	0.985				0.938							
	II		0.936				0.712						
	III			0.907				0.795					
	IV				0.904				0.910				
AspaT	I	0.943											
	II		0.848										
	III			0.954									
	IV				0.925								

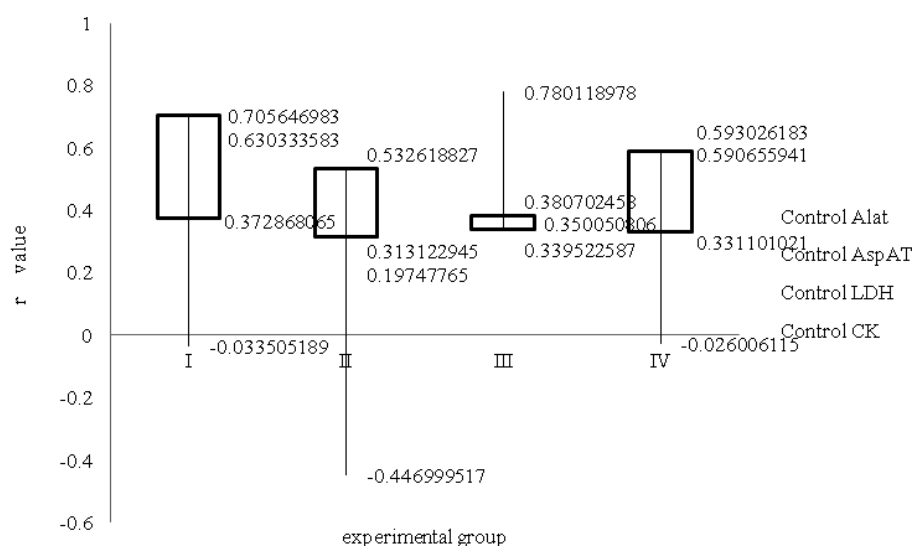

Fig. 5. Value of the correlation coefficient between the experimental and control groups

disease states that involve damage to the myocardium, red blood cells, kidneys, skeletal muscle, liver, lungs, and skin (18). The significant increase in LDH activity in group I can be linked to interaction between inflammatory mediators and increase in anaerobic glycolysis, which is characteristic of the ischemic syndrome in the context of the development of MODS. An increase in the activity of this enzyme observed after the burns indicates advanced anaerobic metabolism in the cytosol of cells with intense energy metabolism (19). The LDH activity recorded in the study groups shows an increase after necrectomy. Similar are the responses of the body after amputations, which are a form of necrectomy, as described in the literature (4, 17, 22, 24). The increase in LDH activity following necrectomy highlights the traumatic nature of the procedure and indicates an increase in anaerobic metabolism as part of the developing MODS, as shown in our study (19). A particular increase in CK activity is noted in organ ischaemia. The activity of CK, a nonparenchymal enzyme, is an accepted parameter of the vascular endothelial damage of parenchymal organs (12, 13). The results obtained in this study may indicate ischemic damage to vascular endothelial cells caused by the generation of high concentrations of ROS, including iNOS, due to the burn wound, as well as trauma, such as postincision necrectomy (3, 8, 10). In the state of ischemia and hence an ATP deficit that accompanies a burn, phosphocreatine, which belongs to phosphagens, becomes an energy source for vascular endothelial cells, and thus an increased phosphorylation of creatine by CK occurs (6). The significant elevation of CK activity in the study groups indicates that necrectomy may cause organ ischemia and endothelial cell damage. This causes an increase in cell membrane permeability and a concomitant increase in the serum activity of indicator enzymes, such as AlAT, AspAT, and LDH. Changes in parenchymal and nonparenchymal enzyme activities are the evidence of trauma, organ dysfunction, and energy disturbance due to developing SIRS and MODS (12). Our results show that a burn alters the activity of both cytoplasmic indicator enzymes (AlAT, AspAT, LDH) and the nonparenchymal enzyme (CK).

The dynamics of AlAT and AspAT in the present study reveal a strong or significant increase in their activity after the burn and a significant decrease after necrectomy in groups II and III ($p < 0.05$). In group IV, decreases in AlAT and AspAT activities after necrectomy were also noted, but were not statistically significant ($p > 0.05$). These observations indicate that necrectomies in groups II (N at hour 12) and III (N at hour 24) reduced the increase in AlAT and AspAT activities. The study showed a very high correlation of changes in parenchymal AlAT and AspAT enzyme activities in all study groups. In group I, a very high positive AlAT/AspAT correlation was noted ($r = 0.943$). However, group II (N at hour 12) showed a significant decrease in AspAT activity to 39.2 U/L ($p < 0.05$) after necrectomy, which persisted until the end of the study. It should be noted that this was the lowest AspAT activity at hour 96 (i.e. at the end of the study) among all study groups. In group II, the AlAT/AspAT correlation was very high ($r = 0.848$). In group IV (N at hour 60), there was a decrease in AspAT activity after necrectomy for 2 study periods. The study showed high positive correlations between CK and AspAT, CK and AlAT, and CK and LDH, which indicates the traumatic nature of necrectomy. The study showed an association between the timing of burn wound necrectomy and changes in AlAT, AspAT, LDH, and CK activities. The results show that the removal of necrosis 12 hours after a burn has the greatest limiting effect on the increase in AlAT, AspAT, and LDH activities. Although necrectomy did not decrease CK activity below that at hour 12, the activity recorded at the end of the study in groups in which necrectomy was performed was significantly lower than it was in group I. Performing necrectomy in the hyperacute phase of the burn disease reduces the activity of indicator enzymes by reducing the influence of inflammatory mediators derived from burn necrosis.

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