Local Injection of Hyaluronidase in Increasing Skin Flap Survival: An Experimental Study

Luiz Alberto Soares Pimentel, MD[1]
Regina Coeli dos Santos Goldenberg, MD[2]

1] Member SBCP - Clínica Luiz Pimentel - Niterói - Rio de Janeiro - Brazil.

2] Associate Professor - Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro - Brazil.

Work performed at Instituto de Biofísica Carlos Chagas Filho, UFRJ, Cidade Universitária, Ilha do Fundão - Rio de Janeiro - RJ - 21940-900, Brazil

Address for correspondence:

Clínica Luiz Pimentel

R. Nilo Peçanha, 59
24210-480 - Niterói - RJ
Brazil

Phone: (55 21) 620-6018

Keywords: Hyaluronidase and skin flaps; skin flaps survival; skin flaps.

ABSTRACT

Previously, clinical observations have suggested that local injection of hyaluronidase (HDL) increase skin flaps survival[23]. We have now extended these observations, analyzing the effects of HDL in rabbit skin flaps. Therefore, dorso-lateral, cranially pedicled skin flaps were treated with 1 ml HDL (200 U/ml, treated flaps*) and compared, after 7 days of surgery, with flaps injected with 1 ml saline (*control 1*) or no injected at all (*control 2*). The efficiency of HDL was confirmed by the percentage of necrosis in the flap area (evaluated by tissue color and capillary filling), which achieved 1.47% ± 2.91, 27.64% ± 25.89 and 30.14% ± 27.96 in the* treated groups, control 1 *and* control 2 *flaps respectively. We conclude that HDL is capable of preventing skin flaps necrosis.*

INTRODUCTION

The survival of large skin flaps is a goal to be reached and many surgical investigators have studied the action of many substances, like vasoactive drugs[6, 9, 10, 11, 14], antioxidants[8], corticosteroids[22] and enzymes including hyaluronidase[16].

Since 1929 hyaluronidase, the "spreading factor", has had its pharmacologic properties thoroughly studied[12, 21]. It has been identified as a mucolytic enzyme, whose main action is depolymerization and hydrolyzation of hyaluronic acid, a polysaccharide which is an essential

component of intercellular ground substance[20].

In the early fifties, hyaluronidase was clinically used for the first time in intravenous high doses to treat patients with cerebral edema[25]. Soon afterwards, the enzyme was used in cases of acute myocardial infarct, apparently acting by reducing intramyocardial edema[18, 19]. In 1988 important actions of hyaluronidase upon extracelular matrix macromolecules were shown in an experimental model using the rabbit skin[15]. In this work, the intradermal injection of the enzyme degraded dermal proteoglycans and, due to the endoglycosaminidase activity, dissociated collagen bundles, which was followed by resynthesis of the initially degraded proteoglycans. On the other hand, the elastic fibers network was not altered.

Recently, the enzyme was also used yet in hypodermoclysis in patients with advanced cancer[5].

Several studies using either subcutaneous or intradermal hyaluronidase in areas or around areas of venous extravasation of toxic substances like nafcilin[26], $CaCl_2$[24], hypertonic saline and sodium tetradecyl-sulfate used in sclerotherapy[27, 28], vinca-alcaloids and another cytotoxic drugs[3, 4] demonstrated the effectiveness of hyaluronidase in preventing necrosis.

In the present paper hyaluronidase was injected directly in the skin and subcutaneous tissue of large skin flaps in an attempt to increase the connective tissue permeability, what was supposed to increase the flow of interstitial fluid to the flap proximal end. This was expected to improve the washout of metabolites from

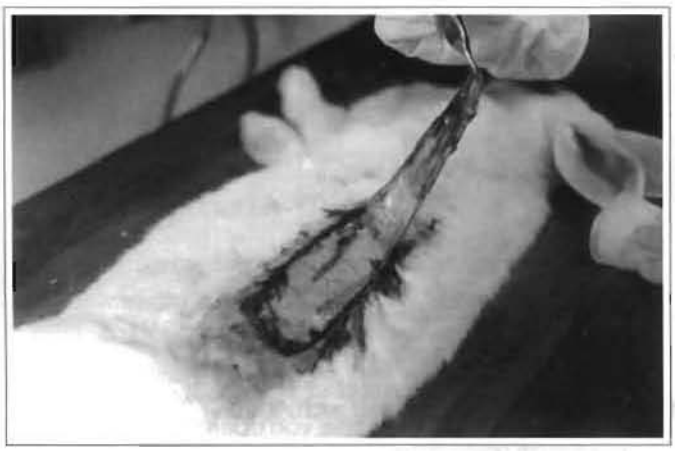

Fig. 1 - The inner surface of an undermined flap.
Fig. 1 - Superfície interna de um retalho descolado.

Fig. 2 - The flap sutured in its bed.
Fig. 2 - O retalho suturado em seu leito original.

Fig. 3 - Injection of HLD solution in the two distal thirds of a *treated flap.*
Fig. 3 - Injeção da solução de HLD nos 2 terços distais de um retalho tratado.

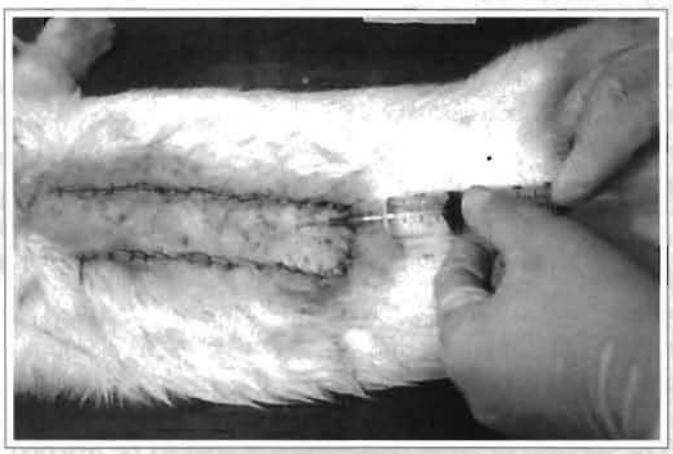

Fig. 4 - Injection of saline solution in a *control-1* contralateral flap.
Fig. 4 - Injeção de soro fisiológico em um retalho controle-1 contralateral.

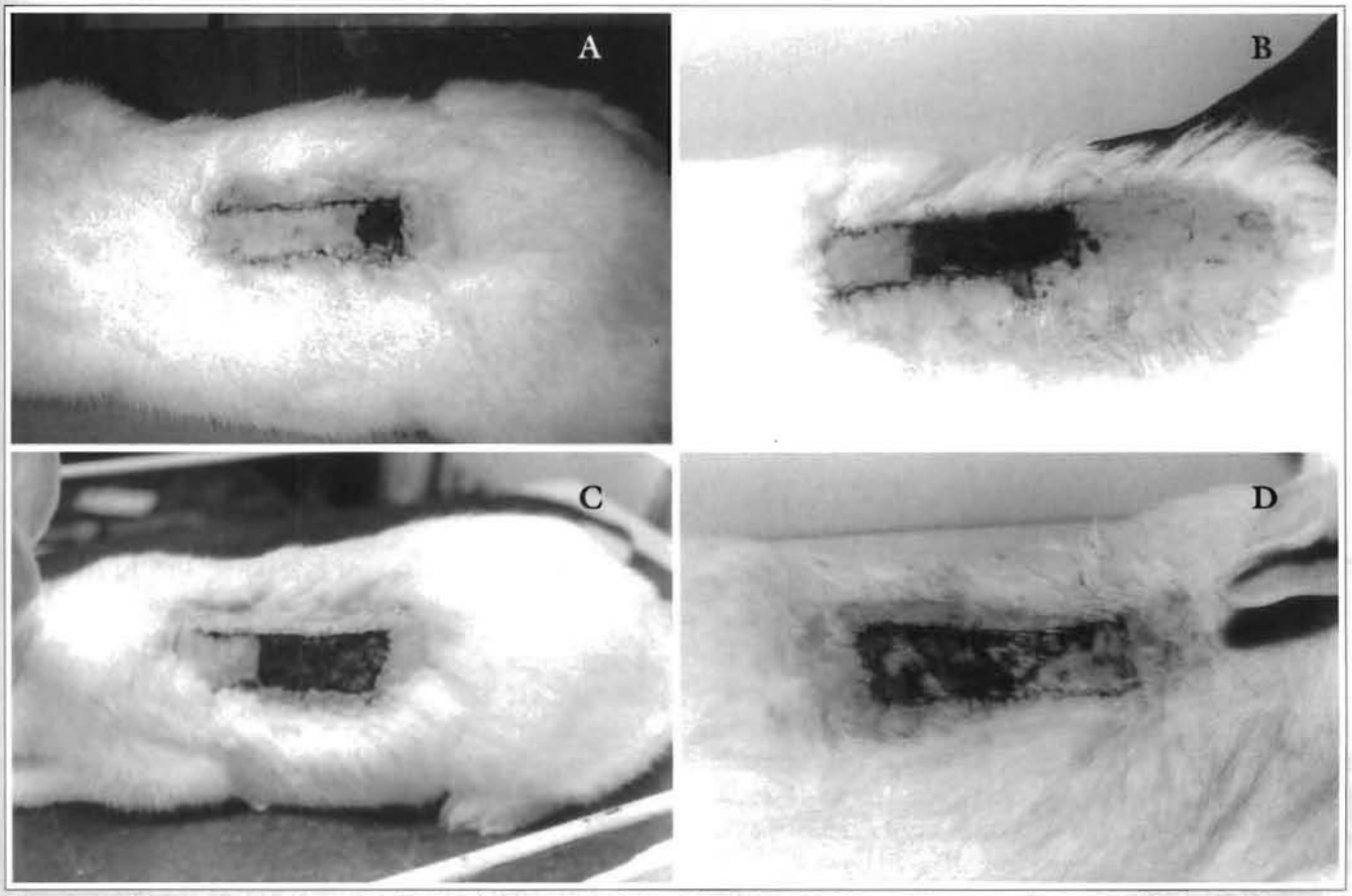

Fig. 5 - Different ranges of necrosis in the distinct control situations: 5a - C1-flap 25 % necrosed. 5b - C1-flap 75% necrosed. 5c - C2-flap 70.3 % necrosed. 5d - Islands of focal necrosis corresponding to 44.4% of a C2-flap.

Fig. 5 - Taxas diferentes de necrose dos retalhos controle: 5a - Retalho C1 com 25 % de necrose. 5b - Necrose de 75 % de um retalho C1. 5c - Necrose de 70,3 % de um retalho C2. 5d - Ilhas de necrose focal somando 44,4 % de um retalho C2.

the flap extremity to more distant areas with normal vasculature, which might lead to prevention of necrosis of the flap. In the studied model, hyaluronidase proved to be an effective treatment in the prevention of necrosis.

MATERIAL AND METHODS

Eighteen New Zeland female rabbits, weighing between 2500g - 3000g were used. They were maintained under equal conditions. Anesthesia consisted of intramuscular administration of ketamine (25mg/kg), diazepan (1mg/kg) and atropine (1ml), followed by shaving of skin and antisepsis with povidine and alcohol. The experimental model was a latero-dorsal randomized skin flap with a cranial pedicle measuring 12.5 cm x 2.5 cm. Each flap was undermined and replaced in its original site and anchored with a continuous 3.0 monofilament nylon suture (Figs.1 & 2).

The study was divided into 2 groups of 9 animals. Group I received two flaps in each animal in the same surgical act. One flap, randomly selected, was injected with the enzyme (HLD 200 U/1ml/day - *treated flaps*) and the contralateral flap injected with saline solution (1 ml/day - *control 1 flaps*) (Figs. 3 & 4). Approximately 1 ml of the HLD solution, was injected over the two distal thirds of the flap divided in 0.1 ml intradermal injections, and in the same manner with the saline solution in the other side. The injections were carried over during seven days, over periods of 24 hours. In group II (*control 2 flaps*) each animal had one flap, observed during seven days without any type of treatment.

The colour and capillary filling of the flaps were daily observed.

On the seventh postoperative day, the necrotic and surviving area were measured.

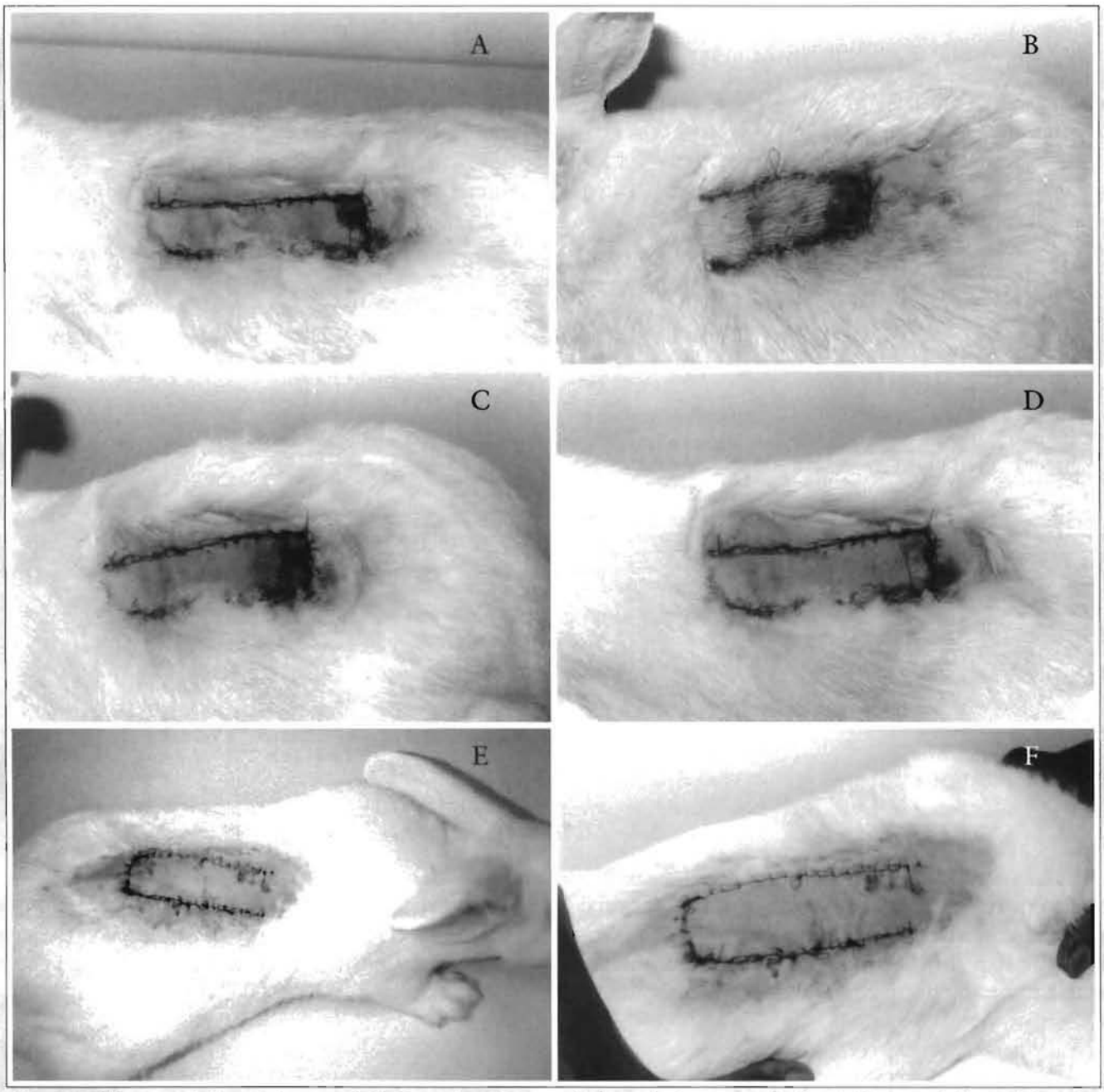

Fig. 6 - Different evolution of the treated (T) flaps : 6a - 6% necrosis showed after one week. 6b - 6.6% necrosis.showed after two weeks. 6c - T-flap suffering distal extremity in the 3rd day. 6d - The same flap of 6c showing color change and complete survival in the 7th day. 6e - Dark color in distal extremity of a T-flap, in the 3rd day. 6f - The same flap of 6e, normal aspect in the 7th day.

Fig. 6 - Formas diversas de evolução dos retalhos tratados (T): 6a - Necrose de 6% no 7º dia pós-operatório. 6b - Necrose de 6,6% após 2 semanas. 6c - Retalho com área de sofrimento na extremidade distal no 3º dia. 6d - Melhora da cor e sobrevivência completa do mesmo retalho de 6c. 6e - Outro retalho T no 3º dia com sofrimento na extremidade. 6f - O mesmo retalho de 6e - normal no 7º dia.

STATISTICAL ANALYSIS

The paired Student's t-test was used for the statistical analysis comparing the difference in survival of the flaps between the *treated* and *control 1 flaps*. In the case of *control 1* vs *control 2* comparison, the non-paired Student's t-test was applied.

Results were expressed as mean ± standard deviation and were considered significant at a 95% confidence level ($p < 0.05$).

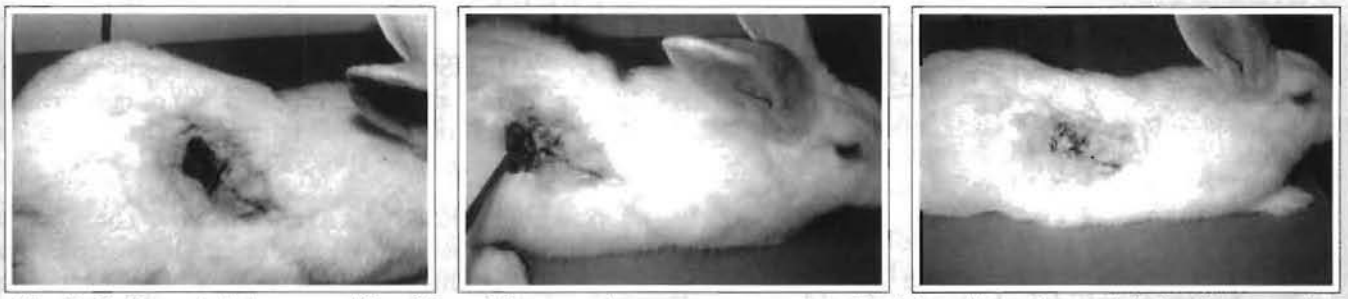

Fig. 7 - Epidermolysis in a treated flap followed by a complete cutaneous recovery: 7a - The epidermal necrosis in its place over the distal third of the flap.7b - The died tissue beeing excised in the 14th day. 7c - The flap with normal skin in its extremity and almost completely cured.

Fig. 7 - Epidermólise seguida de cura completa em um retalho tratado: 7a - A epiderme necrosada no terço distal. 7b - A crosta sendo retirada no 14º dia.7c - Epitelização normal do terço distal já quase totalmente curado.

RESULTS

The aim of this work was to evaluate the effects of hyaluronidase on the survival of skin flaps.

For this purpose, controlled experiments were carried out in rabbits in which large skin flaps were injected with fixed concentration of the enzyme and checked at variable periods of time for the presence of necrosis.

Different non-paired control conditions were adopted: flaps injected with saline, named *control 1 flaps* (or C1), and non-injected flaps, referred to collectively as *control 2 flaps* (or C2).

Both necrosis incidence as well as necrosis extension were undistinguishable between C1 and C2, as presented in Table I and exemplified in (Fig. 5).

In experiments in which flaps were treated with HLD (paired with C1 and C2) incidence as well as the extent of necrotic lesions were dramatically decreased as exemplified in Fig. 6 and represented graphically in Fig. 8 (data in Table I).

Only two rabbits out of 9 tested presented necrosis of their third ending, in approximately 6% of its length (Fig. 6). In additional cases, color alterations reversed, followed by flap survival and recovery during the first week (Fig. 6). Epidermolysis, commonly taken as a superficial necrosis, was observed once in treated flaps, and followed by a cutaneous recovery after two weeks (Fig. 7).

Table I

Animal	%Necrosis* Control 1(C1)	%Necrosis Treated (T)	%Necrosis Control 2 (C2)
1	33.3	6.6	0
2	55.5	0	70.3
3	25	0	33.3
4	75	0	0
5	30	6.0	0
6	0	0	20
7	0	0	70
8	0	0	44.4
9	30	0	33.3
X ± SD	27.64 ± 25.89	1.47 ± 2.91**	30.14 ± 27.96

Necrosis Levels in Treated and Control Groups 1 and 2
*C1 groups represent paired controls for treated flaps.
**Student t-test; statistical significance for $p \leq 0.05$ (null hypothesis).

DISCUSSION

Local necrosis is known to represent a common consequence of plastic surgery. Although efforts have concentrated on investigation of possible pharmacological and/or surgical alternatives to circumvent it in different experimental and clinical models, as confirmed in the scientific literature[1, 2, 7, 13, 17], a safe and efficient maneuver able to prevent acute necrosis of large skin-flaps is yet to be developed.

Hyaluronidase is an enzyme that reduces or prevents tissue injury presumably by causing the rapid diffusion of extravasated fluids to distant areas[3, 4, 24, 26, 27, 28], thus allowing a better turnover of nutrients. The rapid diffusion of fluids is been attributed to a temporary degradation of tissue cement by hyaluronidase[26], leading to a capillary and intersticial leakage of nutrients and metabolites and increasing the rate of cell nutrition.

Our study analyzed the effects of hyalu-

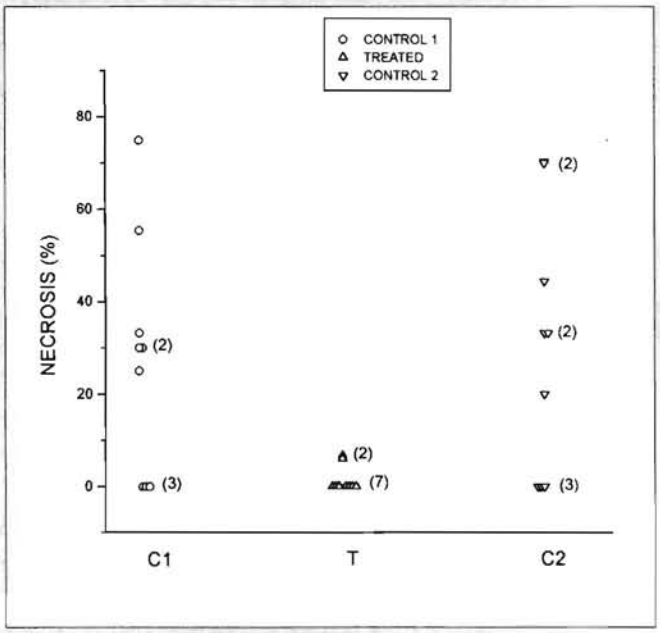

Fig. 8 - Graphical representation of the necrosis extension, experimentally determined in HLD - treated flaps (T), in saline injected (C1) and non-injected (C2) controls. Parenthesis present the number of flaps. Necrosis is virtually absent in treated flaps, as judged by the relatively high and comparable levels of necrosis in both control conditions (C1 and C2).

Fig. 8 - Representação gráfica da extensão de necrose, experimentalmente determinada nos retalhos tratados com HLD (T), nos injetados com soro (C1) e nos controles não injetados (C2). Os parênteses mostram o número dos retalhos. A necrose está virtualmente ausente nos retalhos tratados, em comparação com as taxas de ambas as condições controle (C1 e C2).

ronidase on rabbit skin flap after 7 days of surgery. The data showed a significant reduction in the incidence of necrosis in the *treated* (hyaluronidase) *group* as compared with the *control 1* (saline) group, suggesting that hyaluronidase was effective in improving flap survival. The possibility that the protective effects could be due to saline itself, currently employed as a vehicle to hyaluronidase,was clearly refuted as no significant necrosis prevention was detected in our experimental model and which prevalence (% necrosis, Table I) was comparable to that determined in the non-injected group (C2).

Moreover, the present experimental results substantiate preliminary clinical observations[23] showing that hyaluronidase was effective in improving the survival of larger skin flaps. Although the biochemical and histological basis for hyaluronidase protective effects are yet to be clarified, we believe that the clinical employment of this enzyme in Plastic Surgery must be regarded seriously.

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