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Local hyperalimentation of open wounds

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SUMMARY

After débridement of necrotized and devitalized tissue in deep burns and large traumatic soft tissue defects, the raw surfaces were covered with separate sheets of viscose cellulose sponge (VCS), each moistened by continuous slow infusion of one of the following solutions: (a) 0·9 per cent NaCl; (b) 10 per cent glucose; (c) Rheomacrodex (dextrane hydrolysate in 0·9 per cent NaCl); (d) 7 per cent Levamin (a mixture of essential amino acids and glycin in 5 per cent sorbitol; (e) a combination of two solutions containing amino acids (Le-7402 A) and glucose, electrolytes and vitamins (Le-7402 B). The cellulose sponges were removed or changed 3–7 days later. Macroscopically, histologically and enzyme histochemically, the most active granulation tissue formation was found under the VCS moistened with amino acid solutions, especially when accompanied by glucose and vitamin supplementation. The results strongly suggest that local cellular hyperalimentation of open raw wound surfaces is possible, permitting a new kind of nutritional support in these patients.

PATIENTS with multiple injuries, those with large third degree burns and those undergoing major operations often need intravenous alimentation. A large portion of these nutrients is utilized in the reparative processes, and a portion for other necessary functions of the organism. The nutrients can reach the reparative area provided that the area is well vascularized. If adequate vascularization is lacking, diffusion from vascularized regions is the only way to reach the site under repair.

If the raw surfaces to be granulated are large, the regenerative capacity of the host may be exceeded, resulting in poorly granulating, easily infected surfaces (Viljanto and Ahonen, 1968). Several methods have been used in order to stimulate the wound base (Chardack et al., 1962; Dressler et al., 1967; Artz and Moncrief, 1969). Recently, viscose celluiose sponge (VCS) has been adopted as a temporary cover for large open wounds of traumatic origin and for burned and debrided areas (Viljanto, 1972). In this work the effect of VCS in stimulating the growth of granulation tissue has been further developed by saturating the sponge continuously with amino acid/vitamin solutions.

Patients and methods

Fifty-eight patients, aged from 2 to 62 years, with large open wounds of traumatic origin or third degree burns have been treated in the Departments of Surgery and Paediatrics, University Central Hospital, Turku, since 1966 with VCS as a temporary cover. The

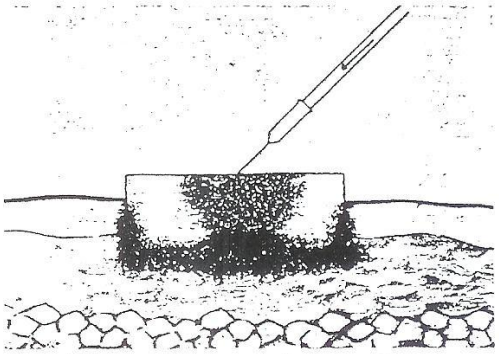

Fig. 1. VCS as a temporary cover for a wound base. Moistening of the sponge by continuous slow infusion.

Table 1: COMPOSITION OF THE SOLUTIONS Le-7402 A AND Le-7402 B

Le-7402 A		Le-7402 B	
Compound	mg	Compound	mg
L-Alanine	307	Glucose	1100
β-Alanine	8	Sodium chloride	6086
DL-α-Amino-butyric acid	34	Potassium chloride	358
		Magnesium chloride	200
L-Arginine	173	Monosodium phosphate	150
L-Asparagine	65	Sodium bicarbonate	2000
L-Aspartic acid	22	Choline chloride	1
L-Citrulline	53	Folic acid	1
Ethanolamine	1	Inositol	2
L-Glutamic acid	86	Nicotinamide	1
L-Glutamine	830	Pantothenic acid	1
Aminoacetic acid	174	Pyridoxin chloride	1
L-Histidine	167	Riboflavin phosphate	1
L-Isoleucine	71	Thiamine chloride	1
L-Leucine	132	Sodium pyruvate	110
L-Lysine	317	Ascorbic acid	550
L-Methionine	32		
L-Ornithine	117	Aq. steril. *ad*	500 ml
L-Phenylalanine	95		
L-Proline	271		
L-Serine	118		
L-Taurine	83		
L-Threonine	194		
L-Tryptophan	98		
L-Tyrosine	91		
L-Valine	199		
Calcium chloride.$2H_2O$	265		
Sodium hydroxide 0·1 M	16·5 ml		
Aq. steril. *ad*	500 ml		

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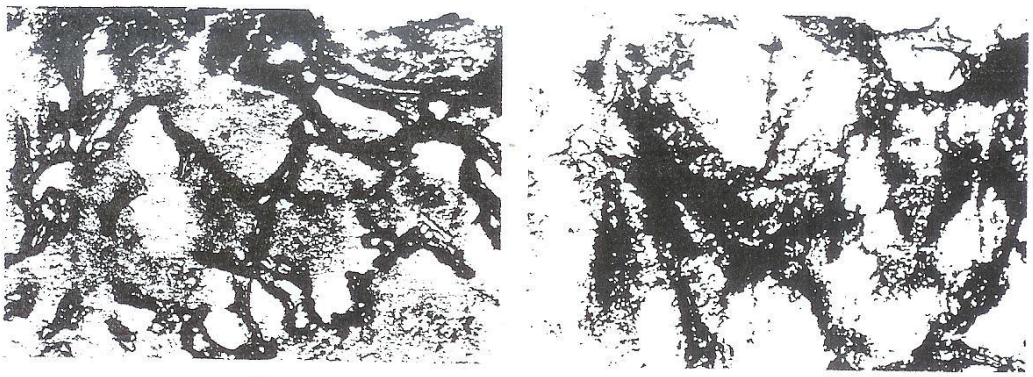
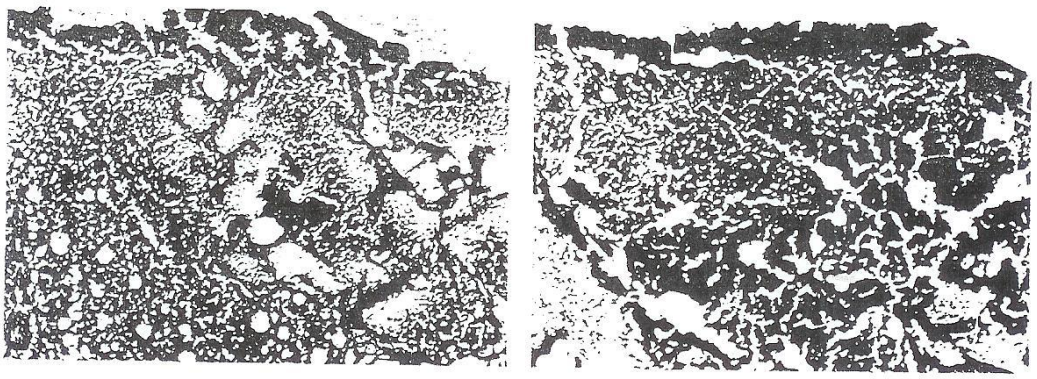


a *b*

Fig. 2. *a*, Adenosine triphosphatase activity of sponge tissue 7 days after the application of VCS to a wound base and moistening with 0·9 per cent NaCl. There is moderate activity, especially near the pore walls. *b*. Adenosine triphosphatase activity at the same time in VCS moistened with Levamin. Note the intense activity near the pore walls. Subject: a boy aged 6 years. AT. (×42.)



a *b*

Fig. 3. *a*, Aminopeptidase activity of sponge tissue after being moistened with 0·9 per cent NaCl for 7 days. The quite high enzyme activity is most pronounced in the zone in contact with the granulating wound surface. *b*. Aminopeptidase activity of the sponge tissue after being moistened with Levamin for 7 days. Note the very intense enzyme activity through the whole tissue, reaching its peak in the zone in contact with granulating surface. Subject: a boy aged 6 years. AT. (×42.)

Table II: ENZYME ACTIVITIES OF GRANULATION TISSUE IN VCS

Enzyme	NaCl 0·9%	Glucose 10%	Rheomacrodex 10%	Levamin 7%	Le-7402 A / Le-7402 B
Acid phosphatase	− − −	− −	− −	+ −	+ −
Alkaline phosphatase	− −	− −	− −	+ − − +	+ + + +
Adenosine triphosphatase	− −	−	− −	+ − +	+ + +
Alpha-esterase	+ −	− −	+	+ − +	+ + +
Aminopeptidase	− − +	− −	+ −	+ − − +	+ + + +
Cytochrome oxidase	− − −	− −	+ −	+ +	+ +
Succinate dehydrogenase	− −	− −	+ − +	+ +	+ +

necessary moisture in the sponge was maintained either by covering the sponge with a cellophane sheet or by continuous slow infusion of saline solution. The 12 most recently treated patients, 5 of whom had sustained necrotizing wringer injuries of an extremity, and 7 with deep burns, were treated with several small isolated sheets of VCS. Each of the sheets was moistened by continuous slow infusion into the centre of the sponge with one of the following solutions (*Fig.* 1): (*a*) 0·9 per cent NaCl; (*b*) 10 per cent glucose; (*c*) 7 per cent Levamin (Leiras, Turku, Finland), a mixture of essential amino acids and glycine in 5 per cent sorbitol; (*d*) 10 per cent Rheomacrodex (Leiras), dextrane hydrolysate, molecular weight 10 000–80 000, in 0·9 per cent NaCl; (*e*) a combination of two solutions Le-7402 A + Le-7402 B containing a mixture of amino acids, electrolytes, glucose and vitamins, specially prepared by the Leiras Company for this purpose (*Table I*). The sheets of VCS were removed after 3–7 days.

After removal of the VCS sheets, the wound bases were evaluated by inspection. Bacterial cultures were taken both from the outer surface of the VCS and from the bleeding wound base exposed. The granulating surfaces were grafted with split-thickness skin immediately after removal of the sponge. If sufficient skin was not available for grafting, as was the case with 2 burned patients, the recipient granulating area was covered again with a new sheet of VCS for the next week or so, until grafting was performed.

The sheets of sponge which had covered the wounds or burns for 3–7 days and which had been continuously moistened by the different solutions were studied histologically. For this purpose, Harris' haematoxylin–eosin and Heidenhain–azan stains were used according to the modifications suggested by Thompson (1966). For enzyme histochemical study, fresh frozen pieces of the sponge were cut at 15 μ in a cryostat and the sections were used for the demonstration of the activities of alpha-esterase, aminopeptidase, succinate dehydrogenase and cytochrome oxidase. To visualize acid and alkaline phosphatases and adenosine triphosphatase, frozen cryostat sections were mounted on glass slides and fixed for 5 minutes in 10 per cent neutral formalin at 4 °C. The sections were further processed as described below (Raekallio, 1970).

Acid and alkaline phosphatase activities were demonstrated by using the azo-dye method of Grogg and Pearse, according to Pearse (1968). Adenosine triphosphatases were demonstrated by using the calcium method of Padykula and Herman, as modified by Novikoff et al. (1961). For alpha-esterase the azo-coupling technique of Gomori was employed, using alpha-naphthyl acetate as the substrate (Pearse, 1972). Aminopeptidase activity was demonstrated by using the azo-coupling method of Nachlas et al., according to Pearse (1972). The Nitro-BT technique of Nachlas et al. was used for succinate dehydrogenase, according to Pearse (1972). Cytochrome oxidase was estimated by the method of Burstone (1959) using *N*-phenyl-*p*-phenyl diamine and 1-hydroxy-2-naphthoic acid as coupling components.

Results

The granulation tissue which had developed under the sponges moistened with amino acid solution showed the most intense reddish brown colour at the wound base as well as in the sponge. The difference was even more pronounced when rather than Levamin a combination of two solutions, Le-7402 A and Le-7402 B, was used. Histological evaluation of the capillary growth and the estimation of the number of fibroblasts, performed as blind tests, showed the greatest values in the sponges which had been continuously moistened with solutions containing amino acids. In these sponges the activities of aminopeptidase, alpha-esterase, alkaline phosphatase and adenosine triphosphatase were the highest (*Table II, Figs.* 2, 3).

In originally clean wounds, bacterial cultures taken from the outer surface of the sponge and from the wound base remained negative. In contrast, sponges applied to infected, débrided burns became contaminated, usually by pseudomonas or proteus bacteria. However, often within the first week of VCS treatment, bacterial cultures taken from the wound base and lower parts of the sponge became negative, indicating that the vital granulation tissue induced by the sponge had overcome the pre-existing infection.

Discussion

The early fibroplasia observed in the sponges moistened with amino acid solutions may be due to a non-specific stimulation of the connective tissue cells at the wound base or due to specific local nutritional support of these cells.

The results of the enzyme histochemical studies give cause for some speculation on the altered metabolism in the wounds treated with solutions containing amino acids. The increased adenosine triphosphatase activity may reflect: (*a*) changes in energy production; (*b*) *de novo* synthesis of enzyme proteins and reparative biosynthesis; (*c*) osmotic activity in nearby vascular structures (Raekallio, 1970). The increased activities of aminopeptidase and alkaline phosphatase are well-known biological properties of proliferating connective tissue cells (Dunphy, 1959; Monis, 1963). The increased activities of adenosine triphosphatase, aminopeptidase and alkaline phosphatase thus reflect the increased synthetic and proliferating functions of the granulation tissue cells stimulated with external local hyperalimentation.

This kind of local temporary treatment with VCS and nutritional solutions offers a new therapeutic approach for the patients whose uneven devitalized soft tissue defect is in need of vital granulation tissue before skin grafting or whose regenerative capacity has been exceeded.

Acknowledgements

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