

# The Effect of a Combined HeNe and I.R. Laser Treatment on the Regeneration of the Lymphatic System During the Process of Wound Healing

P.C. LIEVENS

Vrije Universiteit Brussel, Laarbeeklaan 103, 1090 Brussel, Belgium

**Abstract.** To study the effect of a combined HeNe and i.r. laser treatment on the regeneration of the lymphatic system during the process of wound healing, we compared the evolution of a surgical induced incision wound in two groups of mice.

In the control group (N = 500) as well as in the test group (N = 100) we studied the evolution of four parameters (adhesion, local oedema, regeneration of the vein and regeneration of the lymph vessel) by means of microscopic observations using a cold light source to illuminate the everted skin of the mice. The wounds in the test group were irradiated twice a day with a combined HeNe and i.r. laser. The adhesion of the scar with the underlying tissues disappeared after 10 days in the control group and after 4 days in the test group. The local oedema disappeared in the test group after 8 days while in the control group it lasted until 10 days. A considerable acceleration of the regeneration of both vein and lymph vessel was seen in the test group.

## INTRODUCTION

It is clinically relevant that, after laser treatment, ulcers and wounds have the tendency to heal quicker than untreated ulcers and wounds. Mester (1) has observed that ulcers of the lower limbs, due to circulation disorders, healed quickly after a series of laser therapy treatments.

Wound healing, however, is a very complex process. Lindsay et al (2) and Gillman (3) concluded that the epidermis is the first tissue that bridges over the wound lumen beneath the clot in incised wounds. The regeneration of the epidermis in such wounds is followed by the regeneration of the connective tissue and angiogenesis. However, the reconstruction of the lymphatic vessels during this process is a rather neglected factor, although the role of the lymphatic system is well defined (4). Better comprehension of the phenomenon of lymphatic reconstruction was due to a large extent to studies of Reichert (5), Clark and Clark (6), Danese (7) and Satjukowa (8).

It is evident that in the mechanism of wound healing the lymphatic system plays a crucial role (9).

It will be demonstrated here that the regeneration of the lymphatic system is one of the keys for explaining Mesters' observations.

Our study compared the regeneration process in two groups: one *without* and another *with* a laser treatment.

## METHOD

Two groups of white mice (Albino Suisse type) were considered; a control group (500 mice; without laser treatment); and an experimental group (100 mice; with laser treatment).

After anaesthesia was administered by inhalation of ether, a standardized incision was made on the abdominal skin on the ventrolateral side in the dorsoventral direction (length = 1 cm). The incision cut the whole thickness of the skin and severed completely the major vein, artery and lymph vessel. The wound was then sutured (with a 5.0 silk suture—Astra 621, HSR 18) and re-examined after varying intervals of time (from 1 day to 6 months after the incision).

In order to allow a re-examination and detailed study of the internal aspect of the wound, the mice were anaesthetized with a subcutaneous Urethane,  $C_3H_7NO_2$ , (25%) solution (this product has no effect on the microcirculation). After a median incision along the linea alba the skin was turned back and pinned on a cork plate. The lymph vessels were visual-

ized by means of an injection with Patent V Blue into an inguinal lymph node.

The skin was humidified with a physiological solution during the whole experiment to prevent it from drying out. The scar was examined both macro- and microscopically.

Four parameters were considered in this study:

- a. adhesion of the scar with the underlying tissues;
- b. local oedema;
- c. regeneration of the venous system; and
- d. regeneration of the lymph system.

These parameters were quantified by a 'yes' (existent) or 'no' (non-existent) statement.

In the experiment group ( $N = 100$ ) the mice had the same surgery, but were treated twice a day with a combined HeNe and i.r. laser. During this treatment the mice were restrained by a mild ether inhalation.

The HeNe laser (632 nm) was a continuous one with a power of 5 mW. The i.r. laser was a gallium arsenide pulsed laser (wavelength of 904 nm and a pulsation length of 200 ns) with a peak power of 6.8 W and an average power of 8.8 mW.

The treatment time per session was 4 min. The frequency of the pulsed i.r. laser was 1000 Hz. The distance of the laser from the wound was 2 cm. The area that was exposed to laser irradiation was  $1 \text{ cm}^2$  so that the irradiance for the HeNe laser was  $5 \text{ mW cm}^{-2}$  and the delivered dose was  $1.2 \text{ J cm}^{-2}$ ; the irradiance for the i.r. laser was  $8.8 \text{ mW cm}^{-2}$  and the delivered dose was  $2.1 \text{ J cm}^{-2}$ .

At different intervals the same parameters

as in the control group were examined and compared with the results of the control group.

## RESULTS

### Evolution of the scar in the control group

In this group the mice did not receive any treatment whatsoever. Immediately after the incision a constriction of the arteries and veins was observed. For at least 1 h after surgery no constriction of the lymph vessel was seen and this remained open, leaking its contents into the wound region.

Oedema in the wound region appeared during this phase.

It should be noted that the arteries presented a vasoconstriction over a much greater length than the veins. This probably explains why the artery is never re-established in its original pattern but always in the form of collaterals.

From the third day the neoformation of the vein as well as the lymph vessel was observed.

After about 5 days some venous capillaries were observed to be bridging over the wound area. The newly formed lymph vessels, however, restored as a very disordered and fragile network. The vessel walls of these lymph vessels were very permeable.

In the newly formed veins a slow circulation was observed, while the calibre of the vein to vein anastomosis (which drained the wound region before the regeneration started) was reduced.

After 15 days the skin returned to its normal appearance. The scar was hardly visible exter-

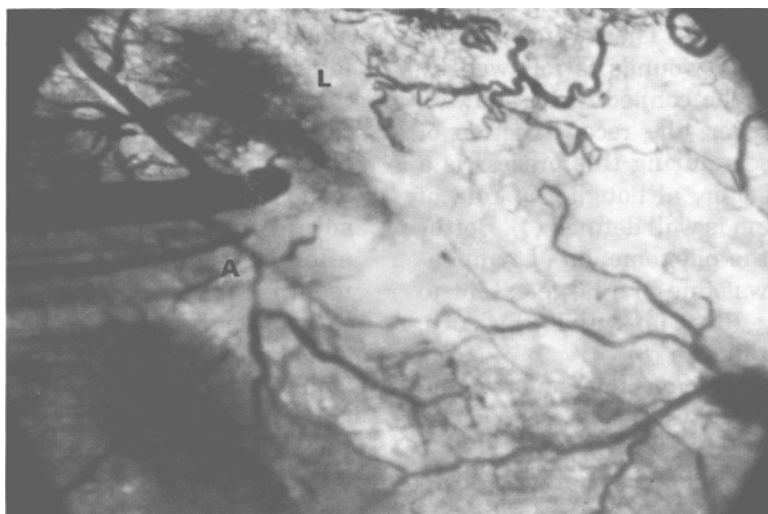
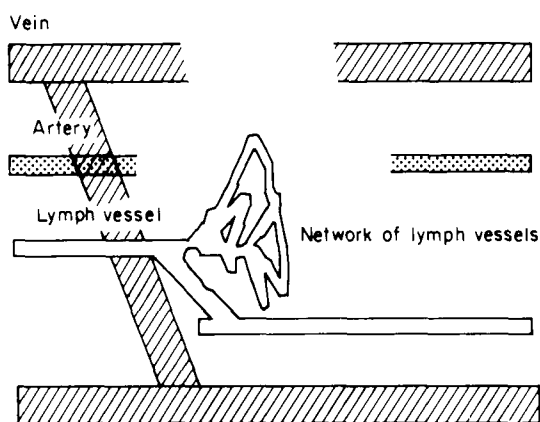


Fig. 1. Leakage of coloured lymph fluid into the wound area. L = lymph vessel; V = vein; A = artery.



nally. The vein was reformed in its original pattern. There is no longer an increase of the permeability of the vein.

However, the reformed lymph vessels remain as a fragile and disordered network which still was abnormally permeable.

Even after 6 months this network of regenerated lymph vessels persisted in the majority of the wounds. Remarkably the preferential lymph flow was via lympholymphatic anastomoses toward the intact side (see Fig. 4).

### Evolution of the scar in the experimental group

The evolution of the four parameters were compared with the evolution of the same parameters of the control group. The data were computerized and the percentage frequencies represented by means of an histogram. The results of the two groups were compared statistically. To detect significant differences we used the Kolmogorov-Smirnov test.

This test can prove whether two cumulative, independent curves are from the same or different populations. When the deviation between the two curves is large enough, we can withdraw the null-hypothesis, which states that there is no significant difference. When we have two cumulative frequency curves with the

Fig. 2. Drawing of the results after 5 days. Venous circulation via venovenous anastomosis, lymphatic regeneration in the form of a network, and lymphatic circulation via lympholymphatic anastomosis.

same intervals, we can calculate the deviations for each interval.

For a well known interval (X) the observed function was  $(y) = Sn_1(X)$  for one curve and for the other  $(y^1) = Sn_2(X)$ . We determined the maximum deviation by making the difference  $0 = \max Sn_1(X) - Sn_2(X)$ . When  $n_1$  and  $n_2$  are bigger or equal to 40 we have a large sample.

For large samples like these the theoretical values can be found in tables. For instance for a significant area of 0.01 the theoretical value is 0.23. This means that when the observed maximum difference is greater or equal to 0.23, we can conclude that with a significant value of 0.01 the curves are from different populations.

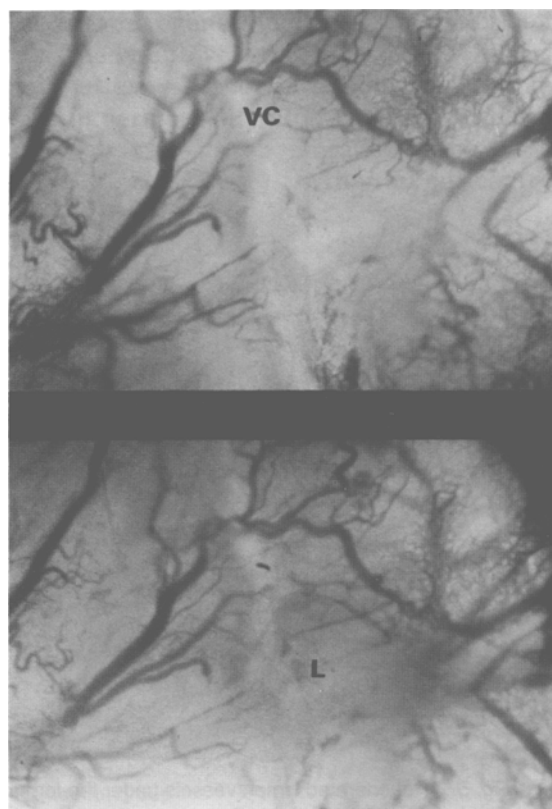


Fig. 3. Fragile network of newly formed lymph vessels (L) 5 days after the incision (magnification  $\times 35$ ). VC = venous capillaries bridging over the wound area.

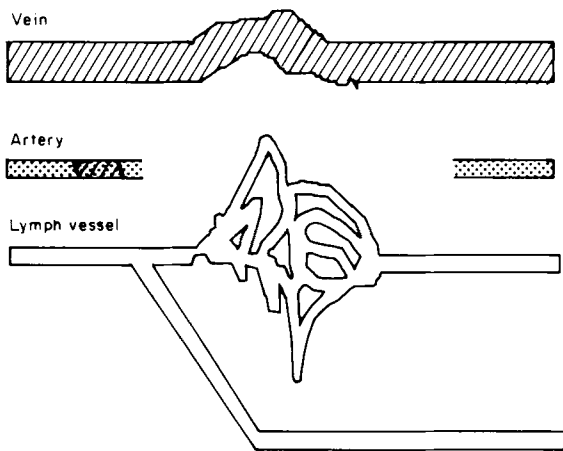


Fig. 4. Drawing of the results after 15 days. Regeneration of the vein, fragile network of new formed lymph vessels, and main lymph flow via lympholympathic anastomosis.

### Adhesion

An important problem in wound healing is the adhesion of the wound with the underlying tissues. This is a phenomenon that in our control group gradually disappeared after 10 days. If this adhesion remains then the wound will evolve into an hypertrophic scar.

In our experimental group this adhesion hardly ever occurred after laser therapy. In the control group it was present in 100% even after the fourth day. Our statistical analysis showed a clearly significant difference (difference matrix = 0.60).

### Oedema

Swelling of the extremities did not appear and general oedema of the abdominal skin quickly disappeared after the incision. Local oedema, however, is a very important factor during wound healing. The existence of this local oedema very often is in correlation with the lack of regeneration of the lymph vessels.

In our experimental group we found that this local oedema totally disappeared after 8 days while in the control group it lasted 10 days. Statistical analysis showed a difference matrix of 0.29.

### The regeneration of the vein

In our control group we found that the vein had recovered in its original pattern after a period of 14 days. After this period the increase in the permeability of the veins in the scar region was

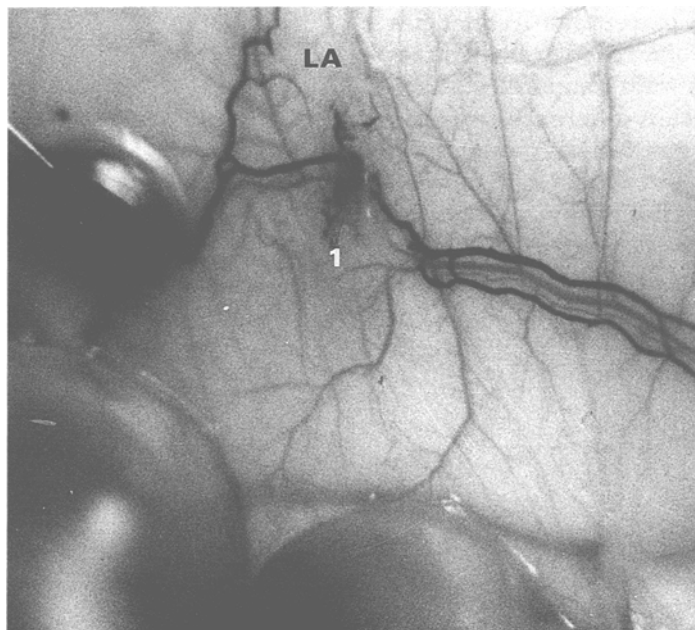


Fig. 5. Regenerated lymph vessels under the forming of highly permeable network 6 months after surgery (1). LA = lympholympathic anastomosis.

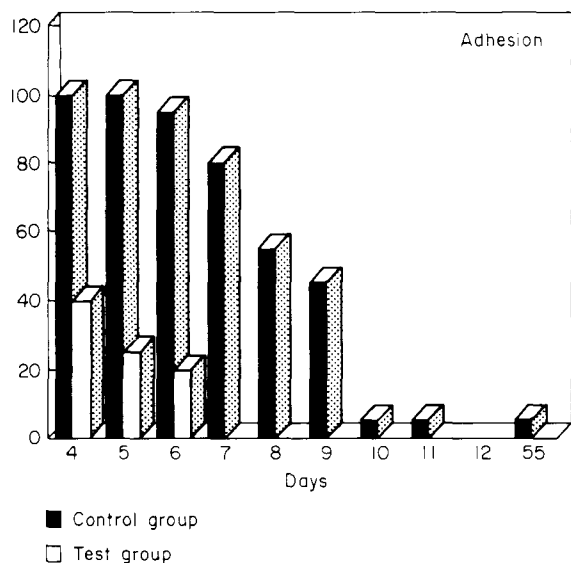


Fig. 6. No adhesion after 6 days in the test group while adhesion persists much longer in the control group.

**Regeneration of the lymph vessel**

In our control group, the lymph vessel was restored in the form of a network of newly formed lymph vessels. The regeneration of the cut lymph vessel appeared in a very specific form which was not seen in cases of regeneration of the veins. A network of small lymph vessels was observed in the wound area after the third day and these vessels were abnormally permeable, so that the injected dye readily left the vessels a few minutes after the injection. This increase of permeability was even seen 6 months later in 50% of the cases.

As in venous circulation reconstruction, we noted that during the lymphatic regeneration the lymph was evacuated by means of lympholymphatic anastomoses until and even after restoration of the original system.

In our experimental group the cut lymph vessel never regenerated in the form of a network. The lymph vessel was restored to its original pattern. We observed hardly any increase in permeability after the first few days. This means that the regenerated lymph vessel was much more functional than in our control

disappearing. Regeneration first appeared in the form of many small capillaries bridging over the wound after 5 days. When the capillaries become functional, the abnormal dilatation of the venovenous anastomosis decreased. After 14 days, one vessel remained functional: this was the elective way of the venous blood circulation. At that point, the other vessels were no longer functional. The flow is reversed at the distal end of the cut vein, going from the wound to the nearest venovenous anastomosis. When the vein had regenerated, the flow became normal again.

During the healing process we also noticed that the blood flow was slowed down in the region of the wound. This phenomenon also disappeared when the normal outflow channel had been re-established. During the healing process dilatation of the veins was also noted. When we compared the rate of regeneration of the veins in our control group and in our experimental group we see that after laser treatment the vein regenerated quicker. Statistically we found a significant difference matrix of 0.47.

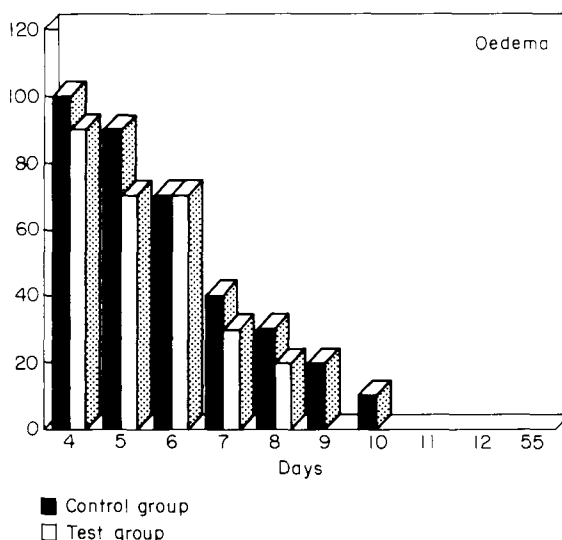


Fig. 7. In the control group oedema disappeared after 10 days while in the test group no oedema was present after 8 days.

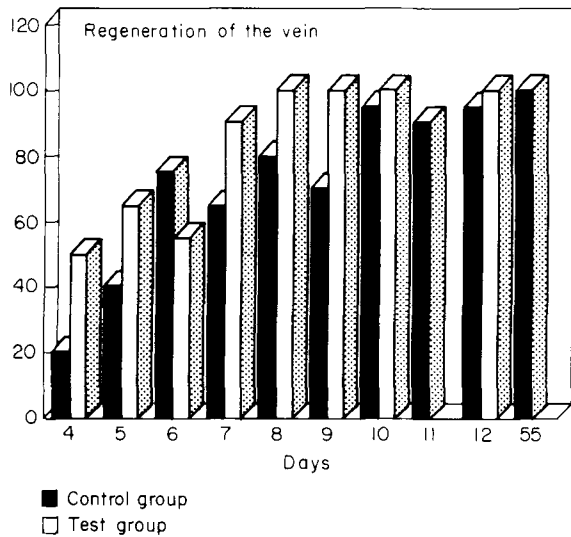


Fig. 8. The vein regenerated quicker in our test group than in the control group.

group. We also observed an acceleration of the regeneration process of the lymph vessel. When we compared the evolution of regeneration in our control group with our experimental group we found a significant difference matrix of 0.45.

**DISCUSSION**

The results from this study confirm what we found in the literature concerning the importance of the lymphatic system during the wound healing process. Clodius (4), Reichert (5), Clark and Clark (6), Danese (7) and Satjukowa (8) have stated that the regeneration of the lymphatic system during the process of wound healing affected the occurrence of oedema and adhesion. The quicker the oedema (fluid waste products) can be drained, the better the wound will heal.

The lymphatic system is primarily responsible for the evacuation of this oedema. If the lymphatic system is destroyed (by the incision) the regeneration process of these lymph vessels will determine the evolution of the scar. In this

study (control group N = 500) we could confirm this statement.

Although no literature was available concerning the role of laser irradiation on this process of regeneration of the lymphatic system, we based our study on the work of Mester (1) who found much better wound healing of leg ulcers after HeNe laser irradiation.

That laser irradiation stimulates the regeneration of lymph vessels was in this study very obvious. However, a lot of research has still to be carried out in order to understand the mechanism of the process.

**CONCLUSION**

In this experiment we have studied the influence of a combined HeNe and i.r. laser treatment on the regeneration process of blood and lymph vessels during wound healing. We compared the evolution of regeneration in a control group and in an experimental group. In both groups we made a standardized transverse incision of the abdominal skin in mice.

For the parameters that we examined: adhesion, oedema and the regeneration of the vein

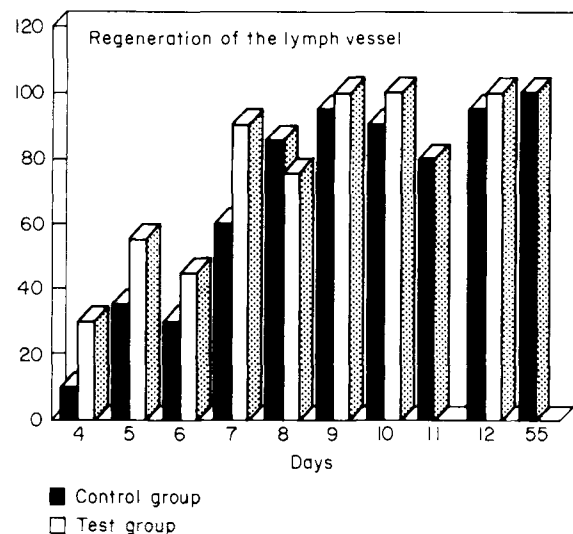


Fig. 9. The lymph vessel regenerated quicker in the test group than in the control group.

and the lymph vessels we can conclude that there is a significant difference between both groups.

Concerning the regeneration of the lymph vessels we found not only an acceleration but also a different form of regeneration. Instead of a network we obtained a regeneration in the original pattern.

We think that these statements can partially explain the better healing of wounds as a result of laser treatment.

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*Key words:* HeNe laser; I.r. laser; Wound healing; Lymphatic regeneration