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THE EFFECTS OF COOLING ON EXPERIMENTAL FREE FLAP SURVIVAL

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Cooling of amputated tissues has been shown to prolong the duration of ischaemia that will permit successful revascularisation. Some authors have suggested that cooling may safely extend the ischaemia time by 24 hours, or more (Malt *et al.*, 1972; McNeill and Wilson, 1970). Successful experimental and clinical replantations of major extremity amputations have been accomplished following 18 to 30 hours ischaemia, using supplemental cooling (Lapchinsky, 1960; O'Brien, 1977).

Hayhurst *et al.* (1974) observed that longer periods of ischaemia increased the damage to cooled monkey digits, especially in the vessels. They suggested that 6 hours normothermic (20–25°) or 24 hours hypothermic (0–4°) ischaemia would not preclude survival of a digit.

May *et al.* (1978) demonstrated 100 per cent survival of experimental free flaps in rabbits following 4 hours normothermic ischaemia. Eighty per cent of the flaps survived after 8 hours of ischaemia. Torii *et al.* (1977) demonstrated a high rate of survival in rabbit flaps after 9 hours of ischaemia at room temperature while storage for 24 hours significantly reduced survival. Histologically, degenerative changes were observed at an earlier time in the muscle, but also occurred in the fat and vessels after 24 hours of storage.

The research project described in this paper was designed to determine the effects of prolonged cooling on revascularised flap tissue. Cold ischaemia time was correlated with the survival rate, the early histological changes after revascularisation and the ultimate quality of the surviving tissue.

MATERIALS AND METHODS

The experiments were performed on New Zealand white rabbits, weighing 2 to 2.5 kg. Anaesthesia was induced with intravenous pentobarbital (30 mg/kg) and maintained with inhalation of halothane, nitrous oxide and oxygen. Both groin areas were shaved and cleaned with chlorohexidine solution. Oval epigastric flaps, measuring at least 2.5 × 3 cm. were raised from each groin. With the aid of a Zeiss Operating Triploscope, the femoral vessels were ligated, just proximal and 1 to 2 cm distal, to the origin of the epigastric vessels. The isolated flaps were completely detached and wrapped in gauze moistened with normal saline solution. They were packed in plastic specimen containers and refrigerated at 6 to 7° for 1 to 6 days. The groin wounds were closed.

After a specified time, the flaps were removed and warmed in normal

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saline solution at 33°C for 5 to 10 minutes. The groin wounds were opened and the flaps were sutured in place, interchanging the flaps from each side. To ensure adequate vessel length, the femoral vessels of the flaps were sutured in a reversed position, using the operating microscope and 10.0 interrupted nylon sutures. There was no adverse effect on the venous drainage as the proximal segment of the femoral vein contains no valves.

Four series of investigations were accomplished utilising 153 rabbit free epigastric flaps. One group of control flaps (10) were studied after cooling only and another normothermic ischaemic (1 hour) group (6) after 1, 2 or 6 weeks revascularisation.

The first group of 82 flaps were cooled from 1 to 6 days. After revascularisation flap survival was determined at 1 week in those flaps which demonstrated venous return (reflow). Arterial anastomoses alone were performed in some flaps demonstrating no reflow. These were defined as those in which no venous blood flow was observed 30 minutes after revascularisation. In most instances, they were considered non-viable, and discarded.

A second group of 35 cooled flaps were revascularised and perfused for 1, 6 or 24 hours. After the specified period, a carbon solution was injected into an ear vein, and the rabbits were sacrificed 5 to 10 minutes later. These flaps were examined histologically for patency of the vessels and the acute effects of ischaemia.

A third group of 20 cooled flaps were examined grossly and histologically after varying intervals of ischaemia. Four flaps cooled for 1 day, and 6 flaps cooled for 2 days, were removed 1 week following revascularisation. Two flaps cooled for 3 days were removed after 2 weeks. Eight flaps cooled for 1 to 4 days were removed 6 weeks later.

RESULTS

Observations in control group of flaps. An apparently normal epidermis was seen in those flaps which were examined after 1 to 6 days cooling and rewarming without revascularisation. The vessels appeared patent and filled with uniform proteinaceous material. The muscle appeared normal. Sebaceous glands in a 4-day specimen demonstrated severe desquamation.

The control flaps, ischaemic for 1 hour at room temperature, demonstrated no significant abnormality on histological examination 1, 2 or 6 weeks after revascularisation (Fig. 1). The skin, fat and muscle appeared viable in all specimens. The most significant change was a reaction at the edge of the fat thought compatible with operative trauma. In a 6 week specimen, one end of the muscle was fibrotic although the remainder of the muscle was viable.

Macroscopic findings in flaps revascularised for 1 week. After cooling for periods of from 1 to 6 days, groups of 12 to 20 flaps were revascularised. Following 1 or 2 days cooling, 31 of 32 flaps survived (97 per cent). Fourteen of the 20 flaps cooled for 3 days survived while 6 either died or exhibited no reflow. Six of the 20 flaps cooled 4 or 5 days survived. Following 6 days cooling, only 1 flap out of 20 survived (Fig. 2).

The quality of survival corresponded to the incidence of viable flaps in each group. At exploration 1 week after revascularisation, flaps cooled for 1 to 2 days demonstrated minimal oedema, a pink colour and good bleeding on incision.

G. 1. A. Top half of control flap left in place 6 weeks. Note normal pattern in underlying fat (H & E 100). B. Photomicrograph of deeper tissues in 6 weeks control flap. Muscle and fat normal. Vessels demonstrate no abnormality (H & E × 100).

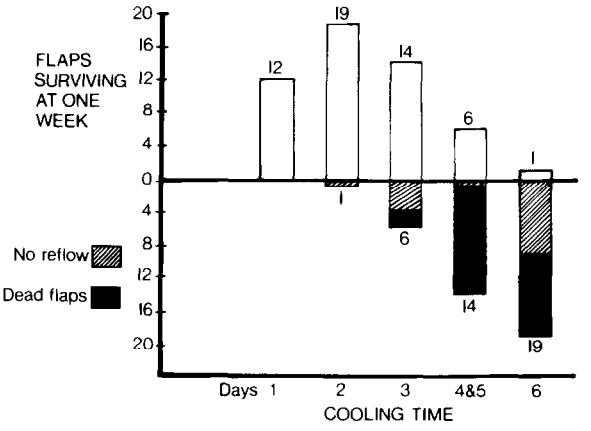

FIG. 2. Survival of flaps at 1 week after cooling 1 to 6 days.

Flaps cooled for longer periods showed more oedema and less vigorous bleeding, although the skin remained normal in texture, colour and appearance. Flaps cooled for more than 3 days were very oedematous and at sacrifice after 1 week the under surfaces were pale and indurated. In non-surviving flaps, although the tissue became necrotic, the anastomoses and the femoral vessels were usually patent and demonstrated no thrombosis. Oedema and thrombosis were often seen in the epigastric and smaller vessels.

Histological examination of flaps revascularised for less than 24 hours. Table I summarises the microscopic changes seen in flaps cooled for 1

TABLE I
Microscopic changes

	Days Cooled		
	1 Day	2 Days	3 Days
Cooled and rewarmed	No change	No change	No change
Revascularised			
1 Hour	-	Inflammation (+) Oedema (+)	Inflammation (+) Venous thrombosis and damage to interior arterial wall (1 case) Oedema (+ +)
6 Hours	Normal skin and fat Inflammation (+) Oedema (+)	Normal skin and fat Inflammation (+ +) Oedema (+ +)	Normal skin and fat Inflammation (+ + +) Dead muscle Oedema (+ + +)
24 Hours	Normal skin and fat Inflammation (+ +) Oedema (+ +)	Normal skin and fat Inflammation (+ + +) Oedema (+ + +)	Skin and fat viable Inflammation (+ + + +) Oedema (+ + + +)

to 3 days and revascularised for 1, 6 or 24 hours. Flaps cooled for 1 to 3 days, reperfused for 1 hour, and injected with carbon prior to sacrifice, appeared normal except for evidence of early acute inflammation and oedema. In one specimen, cooled for 3 days and revascularised for 1 hour, venous thrombosis and inflammation in an arterial wall was present. More extensive inflammation and progressive oedema were exhibited in flaps reperfused for 6

hours with the addition of carbon prior to sacrifice. Early inflammatory changes were seen in the flaps cooled for 1 day. Flaps cooled for 2 days demonstrated more extensive inflammation while flaps cooled for 3 days exhibited dead muscle as well. Carbon was visible in the small vessels of all the 6-hour specimens.

The most severe changes were observed in the flaps cooled for 3 days and reperfused for 24 hours. Inflammation and oedema were maximal at that point; the skin and fat, although abnormal, was viable. Inflammation in the 1 and 2-day flaps, revascularised for 24 hours, was less severe than in 3-day specimens, but more advanced than in the 1 and 6 hour revascularised flaps.

As shown in Table I, oedema and inflammation increased with the duration of revascularisation and the number of days for which they had been cooled. All the flaps were considered to be viable on the histological findings.

Examination of revascularised flaps at 1, 2 or 6 weeks. Flaps cooled for 1 day, revascularised, and examined after 1 week, showed some viable muscle and moderate inflammation of the fat with normal epidermis. Flaps cooled for two days and reperfused for one week demonstrated severe inflammation of the fat with granulation tissue formation and fibrosis. The epithelium, again, appeared normal. After 2 weeks, flaps cooled for 3 days prior to revascularisation showed progressive, extensive inflammation, and fibrosis of the fat. No viable muscle was demonstrated (Fig. 3). In all these flaps the skin appeared viable and the vessels were patent.

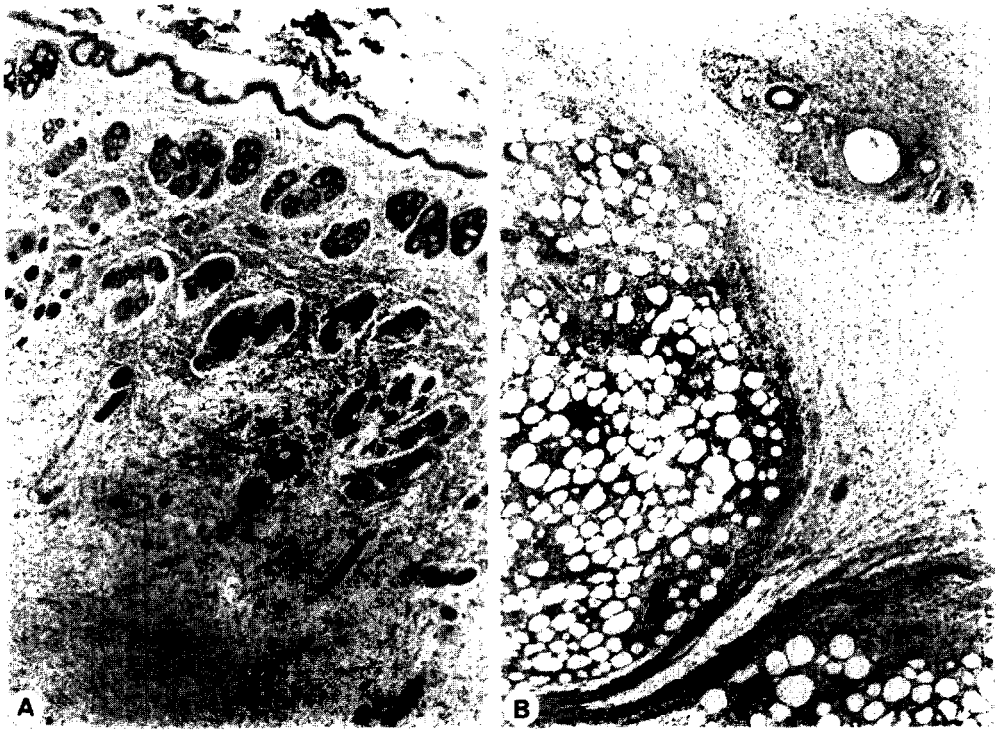

FIG. 3. A. Epithelium and underlying tissue of a flap cooled 3 days, revascularised 2 weeks (H & E × 60). B. Extensive inflammation and early fibrosis in fat (H & E × 60).

A group of 8 flaps were cooled for 1, 2, 3 or 4 days and left in place for 6 weeks. On gross examination 6 weeks later, the flaps exhibited a progressive increase in the quantity of fat necrosis. Under a 1-day flap, only a few small scattered areas of fat necrosis were present; whereas, a 4-day flap contained a large mass of firm fibrotic fat. However, histological examination revealed no significant difference in the 1, 2, 3 and 4-day cooled revascularised flaps. There was generalised fibrosis in the fat and dermis with some residual inflammation. No viable muscle was present. The epidermis was viable; the small vessels were patent and demonstrated no significant abnormality (Fig. 4).

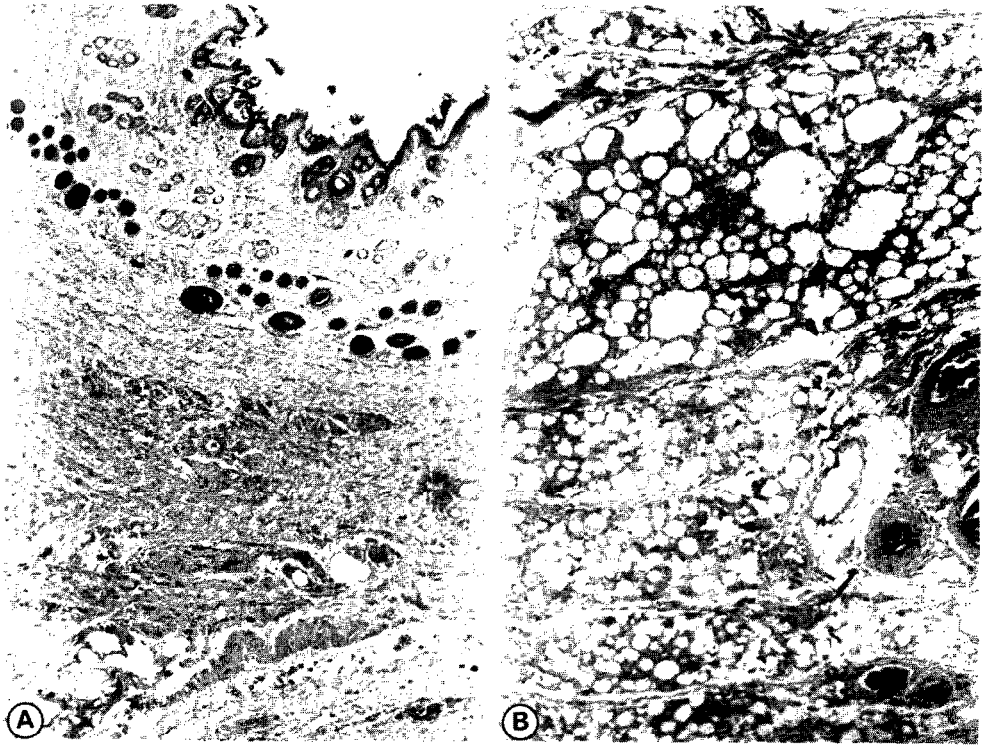

FIG. 4. A. Surface portion of a 3-day cooled, 6 week revascularised flap (H&E × 60). B. Residual inflammation and fibrosis (H&E × 60). (Compare to Figure 3. B.).

DISCUSSION

In this experimental model, cooling of free flap tissue to 6–7°C significantly increases the ischaemic time prior to successful revascularisation. Eighty-six per cent of the flaps cooled for a period of 1 to 3 days survive.

A relationship appears to exist between the length of cold ischaemia and the quality of surviving tissues. At 1 week post revascularisation, less induration and better vascularisation are clinically apparent in flaps cooled for 3 days or less than in those with a longer ischaemia time. The incidence of fat necrosis is also increased with duration of ischaemia. Dead flaps demonstrate marked oedema and scattered thrombi in peripheral vessels, but in general

with microvascular anastomoses the large vessels supplying the flaps are patent.

Similarly, histological changes in 1 to 3-day cooled flaps reperfused for 1, 6 or 24 hours, increase markedly in severity with the duration of cold ischaemia and the period of revascularisation. Three-day cooled flaps 24 hours post revascularisation demonstrate severe oedema and extensive acute inflammation especially in the fat. No viable muscle is observed in any 3-day cooled specimen. These changes are thought to represent the metabolic deprivation in the ischaemic tissue with the subsequent death of some cells in the flap, primarily muscle and fat. The epidermis remains viable and most small vessels within the flap demonstrate little abnormality although in one flap, the vessels became thrombosed and there was inflammation in an arterial wall.

These changes lend support to the hypothesis of the ischaemia-induced no-reflow phenomenon (May *et al.*, 1978). Ischaemic injury induces progressive cellular swelling, intravascular aggregation and leakage of intravascular fluid to the point that adequate tissue perfusion ceases and the flap dies. The presence of patent non-thrombosed primary main flap vessels and anastomoses would indicate that first perfusion of the flap ceases (cellular death), and then thrombosis of the flap vessels occurs.

The tissues within the flap exhibit varying sensitivities to cold ischaemia. The most significant changes were observed in the muscle, followed by the fat. The epidermis and small vessels within the flaps show few histological changes. Although the skin in many of these flaps survives, the underlying muscle, fat, and connective tissue are damaged and partially replaced by fibrous tissue.

The reaction to cold ischaemia injury is maximal in the flaps revascularised for 2 weeks. The inflammatory changes and fibrosis are indistinguishable 6 weeks after reperfusion in the 1, 2 3 and 4-day cooled flaps. Some of the changes in the fat and dermis appear to resolve in the 6-week specimens, especially in those flaps cooled for only 1 or 2 days. No normothermic revascularised control flap, ischaemic for less than 1 hour, exhibits any significant abnormality other than operative trauma near the wound edge.

The value of cooling is well-known in replantation surgery and is an integral part of the protocol in every case. Cooling allows time to prepare the patient and organise the operating theatre and staff for a microsurgical procedure. Despite the clinically proven effectiveness of cooling, digits are occasionally lost due to prolonged warm ischaemia with multiple digit replantation. A well-vascularised replant may become markedly swollen, discoloured and turn dark and necrotic despite a technically well-executed procedure. The simple maintenance of cold ischaemia would have resulted in a viable functioning digit.

Cooling is also applicable in elective procedures. Anderl (1977) reported the storage of a groin flap for 24 hours when spasm in a tibial artery could not be relieved. The flap was later revascularised and survived completely.

This experimental model may be useful in studying other methods of decreasing inflammatory changes, tissue fibrosis and the likelihood of cell death. Ultimately it is possible that prolongation of the ischaemia time could well improve the quality and quantity of tissue that can be successfully removed and transferred as a free flap.

SUMMARY

Cooling to 6–7° significantly increases ischaemic tissue survival to 48 and, probably, 72 hours. Inflammatory changes develop in direct proportion to the duration of the cold ischaemia time. The skin and vessels within the flap appear to be minimally affected by cold ischaemia while fat and muscle demonstrate significant necrosis, inflammation and ultimate fibrosis. Very little, if any, muscle survives the ischaemic insult studied in this model.

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