

## **Chapter 8: Skin Flap Physiology**

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The creation of a cutaneous flap applies specific stresses to otherwise normal skin. These stresses include local tissue trauma and reduced neurovascular supply to the affected tissue. The extent to which skin can survive these injuries is a reflection of the anatomy and physiology of skin as well as the cutaneous response to injury. Knowledge of these principles has led to improvement in skin flap survival by means of flap design and flap delay. Further attempts to augment cutaneous flap survival have been directed at taking advantage of cutaneous physiology by minimizing the deleterious effects of flap creation and combating the metabolic and cellular events that ultimately lead to tissue death.

### **Anatomy**

#### **Skin (Fig. 8-1)**

The epidermis of the skin is derived from ectoderm in the early embryo. The glandular appendages of the skin (sebaceous glands, hair follicles, etc) develop from tubes and solid cords that invaginate from the covering ectoderm (Langman, 1975). The epidermis is made up of stratified squamous epithelium that consists of two categories of cells. The majority of cells undergo keratinization and form the various epithelial layers. The superficial keratinized cells of the skin are replaced continuously by cells arising as a result of the mitotic activity in the basal layer of the epidermis (Bloom and Fawcett, 1975). Melanocytes derived from neural crest cells are also found in the epithelium of skin and comprise a second cell type.

The dermis is derived from embryonic mesoderm and has an average thickness of 1 to 2 mm (Bloom and Fawcett, 1975). The outer surface of the dermis has an uneven border contacting the epidermis and is known as the papillary layer. The remainder of the dermis is called the reticular layer.

Deep to the reticular layer of the dermis the anatomy of loose-skinned and fixed-skinned animals diverges. In fixed-skinned animals (man and swine), the subcutaneous layer consists of loose connective tissue and a varying amount of fat cells and is a deeper continuation of the dermis and collagenous fibers continuous with those in the dermis (Bloom and Fawcett, 1975). The density of the collagenous fibers is related to the degree of cutaneous mobility over the underlying structures. In the palms and soles, for example, these fibers are particularly numerous. The deep surface of the subcutaneous layer is attached to the superficial fascia of underlying muscle where it is present.

In loose-skinned animals (rat, rabbit, and dog) the panniculus carnosus muscle is firmly attached to the reticular dermis and is separated from the superficial fascia of underlying muscles by a loose areolar tissue layer. This layer allows for increased mobility of the superficial cutaneous-panniculus carnosus complex relative to the underlying tissue. This mobility afforded by the loose areolar tissue layer creates a greater dependence on direct cutaneous arterial supply than is seen in man.

## **Neurovascular supply to skin**

The arterial supply to the skin can be divided into three functional units (Daniel and Williams, 1973): segmental vessels, which function to distribute blood from the aorta to the undersurface of muscle; perforating vessels, which provide nutritional support to muscle; and cutaneous vessels, which allow for thermoregulation and nutritional support of skin (Fig. 8-2).

The segmental vessels originate as branches of the paired dorsal aortas during the embryologic period (Langman, 1975). Characteristics of the segmental vessels include (1) a perfusion pressure closely related to that found in the aorta, (2) a location deep to muscle, and (3) a common association with a nerve and vein (Daniel and Williams, 1973).

The perforator vessels are branches of the segmental vessels. These vessels travel via one of two main routes to terminate in the cutaneous circulation. Musculocutaneous arteries pass through the overlying muscle to which they provide nutrition whereas direct cutaneous or septocutaneous arteries travel through fascial septa dividing muscular segments (Daniel and Kerrigan, 1990).

The cutaneous portion of direct cutaneous (septocutaneous) arteries typically runs parallel to the skin surface providing nutrition to a large area of skin. Direct cutaneous arteries typically are accompanied by a pair of veins and run above the superficial muscular fascia (Webster, 1937). The more common musculocutaneous arteries leave the muscle and directly penetrate the subcutaneous tissue to supply a smaller region of skin.

Both direct cutaneous and musculocutaneous arteries empty into a diffuse, interconnecting vascular network often referred to as the dermal and subdermal plexi. This network provides a redundancy in the vascular supply to the skin with the formation of collaterals at the periphery of the vascular territory formed by each musculocutaneous artery. The cutaneous microcirculation consists of a nutrient capillary network in the reticular dermis and arteriovenous shunts in the more superficial papillary dermis (Sherman, 1963). Arterioles, which act as preshunt and precapillary sphincters, regulate the flow through each vascular network (Greene, 1962). Lymphatic vessels form a plexus running parallel and deep to the network of blood capillaries (Bloom and Fawcett, 1975). The lymphatic capillaries end in blind sacs and conduct extracellular fluid back into the bloodstream.

The neural supply to the skin originates from both sensory and sympathetic nerves. The sensory nerves are distributed in segmental fashion, forming dermatomes, and participate in the skin's protective function. The postganglionic terminals of cutaneous sympathetic nerves contain the neurotransmitter norepinephrine and are found in the area of cutaneous arterioles (Anden et al, 1969; Guyton, 1976; Mellander and Johansson, 1968).

## **Skin Physiology**

The skin serves as a sensory and a protective organ. The thick epidermal layers are largely impermeable to gases and to most liquids. Because of this, many agents that could have beneficial effects are ineffective when applied topically to intact skin. Preservation of sensation in transferred cutaneous flaps is desirable, but its effects on the physiology of flaps is unclear.

The blood supply to the skin serves two important functions: it provides nutritional support and is a thermoregulatory mechanism for the body. Primarily because of its thermoregulatory function, the rate of blood flow through the skin is one of the most variable in the body. Under ordinary skin temperatures the amount of blood flowing through the skin (0.25 L/m<sup>2</sup> of body surface area) is approximately ten times the flow required for nutritional support (Guyton, 1976). Blood flow can increase up to seven times this value with maximal vasodilatation. When the body is exposed to extreme cold, blood flow can be reduced to levels that are marginal for cutaneous nutrition.

The two vascular patterns found in skin, the nutrient capillary network and arteriovenous shunts, are integral in performing the two functions of cutaneous circulation. The amount of blood flow to the skin depends ultimately on arteriolar pressure and flow. Under conditions of adequate systemic vascular pressure, however, the distribution of the cutaneous blood flow is regulated by precapillary and preshunt sphincters (Greene, 1962).

The sphincters in the two vascular systems respond to different stimuli. The precapillary sphincter, which controls the amount of nutritive blood flow to the skin, responds to local hypoxemia and increased metabolic byproducts by dilatation (Grange et al, 1976; Wideman et al, 1976). Under such conditions the blood flow is increased (reactive hyperemia being an example). The preshunt sphincters are involved in regulating the changes in blood flow that affect thermoregulation and systemic blood pressure (Folkow, 1960; Sherman, 1963). Release of norepinephrine by the postganglionic sympathetic fibers results in contraction of the preshunt sphincters, diverting blood away from the skin surface where heat loss can occur. With increased body temperature, the sympathetic vasoconstrictor impulses decrease allowing for increased blood flow to the skin (Guyton, 1976).

"Active" vasodilation can also occur with excessive body temperature. Local secretion of acetylcholine by sympathetic nerve fibers, either directly affecting vasodilator fibers or acting through the release of the potent vasodilator bradykinin from the sweat glands, may be responsible. The cutaneous circulation is also extremely sensitive to circulating norepinephrine and epinephrine. Thus, even in areas of skin that have lost their sympathetic innervation, a mass discharge of the sympathetic system will still result in intense vasoconstriction in the skin (Guyton, 1976).

### **Classification of Flaps**

Improvement in skin-flap survival has resulted from improved flap designs that take advantage of the vascular anatomy. Adequate blood flow is so critical to survival that cutaneous flaps have been classified according to their blood supply (Daniel, 1975; Kerrigan et al, 1986) (Fig. 8-3).

#### **Random cutaneous flaps**

The blood supply to a random cutaneous flap is derived from musculocutaneous arteries near the base of the flap. Blood is delivered to the tip of the flap via the interconnecting subdermal plexus in the pedicle. The random cutaneous flap is commonly used in local reconstructions and can be rotated, transposed, advanced, or tubed.

Length-to-width ratios of random cutaneous flaps have been recommended for various areas of the body. These differences reflect a regional variation of the neurovascular supply to the skin. Such a description can serve as a guide in designing random cutaneous flaps (Cook, 1986), but should not imply that a wider flap would extend survival length (Daniel, 1975).

### **Arterial cutaneous flaps**

Arterial cutaneous flaps (also called *axial pattern flaps*) typically have an improved survival relative to random cutaneous flaps. This advantage results from the incorporation of a direct cutaneous artery (recently classified as a *septocutaneous artery* by Daniel and Kerrigan, 1990) within its longitudinal axis. An island flap is an arterial flap with a pedicle consisting of nutrient vessels without the overlying skin. Island flaps can be useful to increase flexibility and reduce pedicle bulk in certain reconstructive procedures.

Use of arterial cutaneous flaps is limited by the availability of direct cutaneous arteries. Examples of arterial cutaneous flaps used in head and neck reconstruction are the deltopectoral flap based on the anterior perforators of the internal mammary and the midline forehead flap based on the supratrochlear vessels.

The surviving length of arterial flaps is related to length of the included direct cutaneous artery. Survival beyond the arterial portion of the flap is based on the subdermal plexus and is essentially a random cutaneous extension of the flap. Flap necrosis secondary to ischemia can be said to occur only in the random portion of the flap (destruction of the arterial pedicle making the entire flap random).

### **Myocutaneous and fasciocutaneous flaps**

Myocutaneous flaps represent an additional modification to improve flap survival. Myocutaneous flaps are based on distal segmental vessels leaving the local vasculature (perforators and cutaneous vessels) intact. This requires incorporating muscle with the flap. Myocutaneous flaps are typically named for the donor muscle. Examples include the pectoralis myocutaneous flap based on the pectoral branch of the thoracoacromial artery and the latissimus dorsi myocutaneous flap based on the thoracodorsal artery.

The increased blood flow and higher tissue oxygen tensions available with myocutaneous flaps (Gottrup et al, 1983, 1984) makes this design superior in the treatment of contaminated or infected defects. Improved phagocytotic and bactericidal activity of leukocytes is seen in myocutaneous flaps relative to random pattern flaps in the canine model (Eshima et al, 1990). These physiologic benefits contribute to the ability of myocutaneous flaps to resist bacterial inoculation more effectively than random pattern flaps.

As is the case with arterial flaps, extending the surface area of the flap is often desirable in clinical situations. A random portion on the flap can be incorporated based on the subdermal plexus. This random extension is usually the portion of the flap most at risk of ischemic necrosis.

Fasciocutaneous flaps use direct arterial (septocutaneous) vessels with the cutaneous branches at the level of the deep fascia, forming a plexus that supplies the subdermal plexus (Cormack and Lamberty, 1984). The appropriate size of fasciocutaneous flaps is less well defined than that of axial pattern flaps with their obvious arterial supply. Fasciocutaneous flaps appear to rely more on potential skin vascular territories. Four types of fasciocutaneous flaps have been described based on the pattern of blood supply incorporated into the fascial component of the flap. Examples include the parascapular flap and the radial forearm flap.

### **Venous flaps**

Flaps with only an intact venous supply demonstrate the minimal nutritional requirements needed for flap survival. In flaps based on the dog saphenous or cephalic vein, survival occurred when the vein was intact on entering and exiting the flap, providing a flow through the venous system (Sasa et al, 1988). Flaps with proximal or distal ligation of the vein or with an arterial pedicle alone necrosed (Amarante et al, 1988; Baek et al, 1985). The surviving flaps showed no evidence of arterial blood flow as measured by injected microspheres until the third postoperative day (Sasa et al, 1988). Venous injections demonstrated little uptake in the flap, but capillary blood flow may not be ruled out in this model (Weinberg, 1988). Venous flaps performed in humans have been most successful in the distal extremities where multiple venous anastomoses and no valves are present (Chavoin et al, 1987).

### **Physiology of Acutely Raised Flaps**

A number of changes detrimental to skin survival occur when a cutaneous flap is created. That flap survival occurs at all is a testimony to the minimal nutritional requirements of skin relative to the blood flow available in intact skin. The primary insult affect flap survival is impaired vascular supply and resultant ischemia. In the presence of adequate blood flow complete flap survival occurs. Nerve section and inflammation can also influence flap survival by affecting blood flow.

### **Vascular**

Partial interruption of the vascular supply to the skin is the most obvious and critical change that occurs with elevation of a cutaneous flap. This interruption results in a local decrease in perfusion pressure to the skin. The decrease in perfusion pressure becomes more pronounced with increasing distance from the base of the flap (Cutting, 1982; Landis, 1927). When perfusion is reduced in one area, the adjacent vascular territories supplied by a separate perforating vessel can provide a low-pressure blood supply via the subdermal plexus. Because the nutritional requirements of skin are relatively low compared to the baseline skin blood flow, a number of vascular territories can be compromised before necrosis will result.

In the arterial or myocutaneous portion of flaps the blood supply is usually adequate (Gottrup et al, 1984) and survival of the cutaneous covering is ensured. The survival length of the random portion of the flap depends on the physical properties of the supplying vessels (intravascular resistance) relative to the perfusion pressure (Daniel, 1975). Nutritional blood flow ceases and flap necrosis occurs when the perfusion pressure drops below the critical closing pressure of the arterioles in the subdermal plexus. In the past random cutaneous flaps

were often designed relative to a desired length/width ratio - a wider base being needed to transfer a longer flap successfully. However, incorporation of additional vessels with the same perfusion pressure by widening the flap does not alter survival length (Daniel, 1975; Milton, 1971).

Myers (1986) has emphasized that "fresh flaps are always both viable and ischemic". Depending on the degree of ischemia and the amount of time before recovery of nutrient blood flow, the flap will either proceed toward necrosis or recovery. In the pig model, arterial and random flaps can tolerate an average of 13 hours of total avascularity and remain viable (Kerrigan and Daniel, 1982b). In the presence of less than total avascularity this period is probably much longer.

In surviving flaps the reduced blood flow gradually increases. If the flap is placed in a favorable recipient site a fibrin layer forms within the first 2 days. Neovascularization of the flap begins 3 to 7 days after flap transposition. Early neovascularization has been detected at 4 days in the pig and rabbit models (Tsur et al, 1980; Verlander, 1964) and at 3 days in the rat model (Gatti et al, 1984). Revascularization adequate for division of the flap pedicle has been demonstrated by 7 days in animal models and man (Cummings and Trachy, 1985; Klingenstrom and Nylen, 1966; Tsur et al, 1980). Distal flap blood flow continues to increase during the second week despite what appears to be an adequate nutritional supply (Cummings and Trachy, 1985; Gottrup et al, 1984).

Ischemia is one of many conditions that can induce angiogenesis (Abrams, 1983; Semashko et al, 1985). Angiogenic agents have been isolated from tumors and multiple tissues (Hom et al, 1988). In the presence of an angiogenic stimulus new capillaries arise from small venules in the recipient site and migrate toward the stimulus. Some capillaries join preexisting flap vessels (inosculation), but the majority of revascularization appears to involve direct ingrowth of recipient vessels into the flap (Smahel, 1977).

The venous outflow from the skin is also impaired with flap elevation. Venous flow can occur through the subdermal plexus or via the single or paired venous channels that accompany the feeding artery in the pedicle. Complete venous occlusion in the early postelevation period may be more damaging to flap survival than inadequate arterial supply. Venous occlusion for 8 hours in the rat island skin flap model was incompatible with flap survival whereas 70% of flaps with a comparable arterial occlusion survived (Su et al, 1982). Fortunately, the subdermal plexus alone is often able to provide adequate venous outflow. Care must be taken, however, to preserve venous outflow in flaps pedicled solely on the feeding vessels.

Impairment of lymphatic drainage with flap elevation also occurs. Reduction of the cutaneous lymphatic drainage results in an increase in interstitial fluid pressure that is compounded by increased leakage of intravascular protein associated with inflammation. The resulting edema formation can decrease capillary perfusion by increasing the intravascular resistance.

## **Nerve section**

Both cutaneous and sympathetic nerves are severed in the process of flap elevation. Although loss of sensation may limit the usefulness of the flap after transfer, adrenergic denervation has implications for flap survival. When a sympathetic nerve is divided, catecholamines are released from the nerve terminal and the mechanism for catecholamine re-uptake is eliminated (Jurell et al, 1968; Palmer, 1970; Pearl, 1981). A local hyperadrenergic state exists, which produces vasoconstriction mediated by alpha-adrenergic receptors in the cutaneous vasculature.

The vasoconstricting effect of sympathectomy further reduces the total flap blood flow (Kerrigan and Daniel, 1984; Pang et al, 1986c), which is already diminished by division of supplying vessels. This negatively affects the ratio of perfusion pressure to the critical closing pressure of the arterioles in the subdermal plexus, and a greater proportion of the distal flap is excluded from the blood supply. The stored transmitter is depleted within 24 to 48 hours (Jurell, 1986; Palmer, 1970) and blood flow increases as the concentration of norepinephrine declines (Pang et al, 1986c). In critical areas of the flap, however, the time to recovery of nutrient blood flow may be delayed sufficiently to produce additional necrosis.

## **Inflammation/prostaglandins**

The surgical trauma associated with an acutely raised flap results in an inflammatory response. Inflammation consists of a vascular and cellular response to injury that prepares the tissue for the repair process. With injury, histamine, serotonin, and kinins are released into the extracellular compartment, markedly increasing the permeability of the microcirculation. The result is an increase in the concentration of proteins and cells within the extracellular space. This response can be beneficial as long as it is limited to nonbacterial inflammation, which begins prior to flap elevation (Liston, 1984; Macht and Frazier, 1980). The inflammation created by flap elevation may be deleterious because of the resultant edema formation.

The action of the primary mediators of the inflammatory response (histamine, serotonin, and kinins) is short-lived. Following kinin formation and in the presence of complement, prostaglandins are synthesized by injured cells. Prostaglandins play an important role in the later stages of the inflammatory reaction while simultaneously initiating the early phases of injury repair.

Prostaglandins are derived from 20-carbon essential fatty acids, which are incorporated in membrane phospholipids (Fig. 8-4). Activation of phospholipases results in the release of arachidonate from cell membrane phospholipids. Once released, arachidonate is metabolized by several distinct microsomal enzyme systems, one of which is cyclooxygenase. The action of cyclooxygenase results in the production of prostaglandin  $H_2$  ( $PGH_2$ ).  $PGH_2$  is chemically unstable but can be transformed into a variety of products.

Prostaglandin  $E_1$  ( $PGE_1$ ) and prostaglandin  $E_2$  ( $PGE_2$ ) can be synthesized from prostaglandin  $H_2$  by isomerases in the vascular endothelium. Both  $PGE_1$  and  $PGE_2$  produce vasodilatation. Prostaglandin  $D_2$  ( $PGD_2$ ) is also formed by an isomerase reaction and is the principal cyclooxygenase product of the mast cell. Its effects on the cutaneous

microvasculature are similar to PGE<sub>1</sub>. Prostacyclin (PGI<sub>2</sub>) is a vasodilating agent and inhibitor of platelet aggregation that is derived from PGH<sub>2</sub> through the action of prostacyclin synthase. In the skin PGI<sub>2</sub> is primarily produced in the endothelial cells of blood vessels (Hauben and Aijlstra, 1984; Kaley et al, 1985). Prostacyclin is metabolized to 6-keto-PGF<sub>1a</sub>.

Thromboxane synthetase converts PGH<sub>2</sub> into thromboxane A<sub>2</sub> (TxA<sub>2</sub>) and is primarily located in the platelets. Its effects include vessel constriction and promotion of platelet aggregation (Kay and Green, 1986). TxA<sub>2</sub> is unstable and rapidly converted into thromboxane B<sub>2</sub> (TxB<sub>2</sub>). Prostaglandin F<sub>2a</sub> (PGF<sub>2a</sub>) is derived from PGH<sub>2</sub> by a reductase reaction. PGF<sub>2a</sub> does not appear to influence blood flow in segmental or perforating arteries but does result in vasoconstriction at these levels. A marked increase in resistance is seen in cutaneous arteries, arterioles, and venules in the presence of PGF<sub>2a</sub> (Nakano, 1973).

The synthesis of prostaglandins and thromboxane can be altered by pharmacologic manipulation. The action of phospholipase A<sub>2</sub> can be inhibited by drugs that reduce the availability of Ca<sup>++</sup>. Glucocorticoids also affect phospholipase A<sub>2</sub> activity by inducing the synthesis of a protein that inhibits the enzyme (Campbell, 1990). Aspirin and other nonsteroidal antiinflammatory medications interfere with the cyclooxygenase enzyme, thus inhibiting the synthesis of PGH<sub>2</sub>.

Recent studies have provided further insight into the activity of prostaglandins in ischemic flaps. Prostacyclin levels were found to increase 4 days after elevation of a porcine flank flap, to peak on day 7, and then to decrease up to postoperative day 21 (Hauben and Aijlstra, 1984). Elevation of a bipediced rat dorsal flap resulted in elevated levels of PGE<sub>2</sub>, PGF<sub>2a</sub>, and TxB<sub>2</sub>, with a return to near-normal levels by day 7. Conversion to a single-pedicle flap ("delay") resulted in a blunted production of thromboxane and an elevated PGE<sub>2</sub> that lasted for at least 7 days. Elevation of an acute flap showed an elevation of PGE<sub>2</sub>, PGF<sub>2a</sub>, and TxB<sub>2</sub> that was greater and more prolonged than seen with surgical delay (Murphy et al, 1985).

Blood samples drawn from a rat hind limb rendered ischemic for 5 hours showed marked elevation of TxB<sub>2</sub>, 6-ketoprostaglandin F<sub>2a</sub> (a metabolite of PGI<sub>2</sub>), and PGE<sub>2</sub> (Feng et al, 1988). A difference between tissue tolerating reflow and tissue demonstrating no reflow was noted. Injection of 2% formic acid into the rat dorsal flap resulted in an increase of TxA<sub>2</sub> and a small increase of PGE<sub>2</sub>. After flap elevation the flaps treated with formic acid demonstrated a decrease in TxA<sub>2</sub> and an increase in PGE<sub>2</sub> (Lawrence et al, 1984). It is clear from these studies that prostaglandins play a role in the inflammatory response after flap surgery. Whether these changes in prostaglandin levels represent a cause or a side effect of the observed phenomenon remains to be demonstrated.

### **Reperfusion (free radicals)**

Return of blood flow to an ischemic flap under the influence of excess vasoconstriction due to excessive release of norepinephrine occurs in approximately 12 hours. With norepinephrine depletion and continued inflammatory response, blood flow can reach a maximum at 24 hours in the rat and pig models (Pang et al, 1986c; Sasaki and Pang, 1980). When oxygen becomes available with reperfusion, an additional menace to flap survival is produced, the free radical. This byproduct of reperfusion can cause damage at both the cellular and subcellular levels, contributing to postischemic tissue necrosis.

Free radicals are extremely reactive compounds by virtue of an unpaired electron in their outer orbitals. Oxygen-free radicals are formed by the sequential univalent reduction of molecular oxygen. The superoxide anion radical ( $O_2^-$ ) is formed by the addition of a single electron to molecular oxygen. Superoxide is a byproduct of adenosine triphosphate (ATP) production in the mitochondria and other oxidation reduction reactions (Southorn and Powis, 1988). Polymorphonuclear cells are a second source of superoxide radicals, which are released in response to bacterial inflammation (Babior et al, 1973).

A major source of free radicals in ischemic tissue is the enzyme xanthine oxidase (McCord, 1985) (Fig. 8-5). With ischemia, high-energy phosphate compounds are converted to hypoxanthine, which accumulates in the tissues. When oxygen becomes available with reperfusion, xanthine oxidase catalyzes the conversion of hypoxanthines into uric acid, producing superoxide in the process. This reaction is thought to be an important mechanism in posts ischemic tissue injury in skin flaps (McCord, 1986).

Xanthine oxidase activity has been found in normal rat skin and increases its activity after venous occlusion and reperfusion (Im et al, 1984). Xanthine oxidase activity also increases after elevation of a dorsal rat flap with the highest levels being present distally (Angel et al, 1988). Tissue damage resulting from free radical production can occur from lipid peroxidation of the cellular membrane and denaturation of the intracellular matrix (Mulliken and Im, 1986; Southorn and Powis, 1988).

### **Research Methods**

A large amount of literature is available on skin-flap physiology. The results of several studies give conflicting results. Experimental results are often difficult to interpret because of variations in choice of animal model, timing of treatment, route of drug administration, method of data collection, and repeatability of the study (Kerrigan and Daniel, 1982a). Some standardization of flap research methods would help resolve some of these difficulties. Guidelines for pharmacologic investigation of skin flaps were suggested by Kerrigan and Daniel (1982a). These recommendations include (1) postoperative treatment only, (2) control flaps on the same animal, (3) baseline fluorescein measurements, (4) double-blind experimental design, and (5) measurement of drug-induced changes in blood flow. No consensus has been reached regarding these or other guidelines.

Two basic experimental designs have been used to investigate the consequences of a vascular insult on a surgical flap. In one design the blood supply to a flap is interrupted for varying amounts of time by occluding or otherwise interrupting flow through the vascular pedicle. The maximum amount of ischemic time the flap can survive in the experimental and control group is determined. This design is useful investigating the no-reflow phenomenon and ischemia tolerance. The second design involves flaps having a random extension in which the effect of experimental manipulation of blood flow or flap survival is compared to a control. From this basic framework a number of animal models and methods to assess blood flow and survival have been developed.

## Animal models

The most commonly used model for flap research is the rat, a relatively inexpensive animal. A large amount of data is available for referencing. Unlike humans, rats are loose-skinned and have a preponderance of skin supplied by direct cutaneous arteries. The abdominal flap is based on the epigastric vessels with an axial pattern on one side and a random extension as it crosses the midline or extends cranially. Petry and Wertham (1984) suggested that some of the survival variance in rat epigastric flaps is caused by an inconsistent incorporation of the lateral branch of the superficial epigastric artery. Dorsal flaps can be based caudad or cephalad. McFarlane et al (1965a) designed a dorsal rat flap so that it became necrotic when raised acutely but survived after a 2-week delay. The amount of necrosis in rat dorsal flaps can vary from 22% to 50% in the cranially based flaps (McFarlane et al, 1965a) and from 30% to 60% in caudally based flaps (Adamson et al, 1967). This variance seen in control animals often means that large numbers of animals are necessary in order to obtain meaningful results.

The pig is another common animal model in flap research. Pigs are fixed-skinned animals with numerous musculocutaneous arteries and their cutaneous blood supply is more similar to that of man. Multiple flaps can be raised, allowing experimental and control flaps to be raised on the same animal. Kerrigan et al (1986) reviewed the flaps available on the pig. Random flaps included the dorsal flank flap, which had a predictable survival, the opportunity for up to 10 flaps per animal, and a position that enabled easy monitoring and care of the flap. Because a variable amount of panniculus carnosus can be included with the flap, it may not be completely random. This situation can be avoided by raising the flap superficial to the muscle. The random buttock flap has the advantage of not having a muscle component, but the experiment is limited to two flaps per animal.

Arterial flaps on the pig flank correspond to the random flaps except that the pedicle is placed ventrally, preserving an arterial pedicle. The ventral flank flap, based 4 cm lateral to the nipple line, has the same advantages and disadvantages as its random counterpart. The arterial buttock flap has a large neurovascular pedicle, no muscle component, a large skin area, and a reliable survival (approximately 13 cm). Again, only two flaps per pig are possible.

The pig is a satisfactory model for studying myocutaneous flaps because of the multiple perforating arteries supplying the skin. A variation of the myocutaneous flap model includes placing a catheter around the pedicle to allow pedicle occlusion at varying schedules (Cummings et al, 1985; Millican and Poole, 1985a). Kerrigan et al (1986) conclude that the latissimus dorsi flap based on the thoracodorsal artery is the best myocutaneous flap model in the pig. The gracilis myocutaneous flap had no reliable necrosis and a poor location. The rectus abdominis myocutaneous flap was faulted for having two dominant pedicles and a dependent position.

A modification for the pig myocutaneous model was suggested by Haughey and Panje (1989). With their design up to 10 myocutaneous flaps are raised on a single animal. Problems with excess skin survival are reduced by limiting the size of the muscle block to 4 x 4 cm. A random extension of skin 12 cm long is created beyond the muscle. A variation among the flaps can occur in that the skin can be loosely attached (gracilis, pectoralis major),

separated by panniculus carnosus and subpannicular fat (latissimus dorsi), or separated by a dense deep fascia (biceps).

The use of flaps created from pig skin to research changes in cutaneous surface area and thickness with tissue expansion has been criticized (Bartell and Mustoe, 1989). The biomechanical properties of pig skin were found to be at variance with human skin. Changes in cutaneous blood flow with tissue expansion are well studied in the pig model.

Rabbits, like rats, are loose-skinned animals. Rabbits have been used as models in skin flap research when a larger skin area is desired in a random flap (Chu and Deshmukh, 1989). Forrest and Pang (1988) found that the skin over the latissimus dorsi muscle was supplied mainly by a direct cutaneous artery. The perforators present were few and could not support the flap. A similar result was found studying the pectoralis major myocutaneous flap (Nieto et al, 1985). The authors of both papers conclude that myocutaneous flap studies in the rabbit may have less relevance to human myocutaneous flaps than equivalent studies in the pig model.

The canine was the first animal model used in skin flap research (Donovan, 1975). A number of studies using the canine model for investigation of flap physiology have been published and are discussed in this chapter. The dog is a loose-skinned animal and care must be used when investigating vascular changes in myocutaneous flaps. The biomechanical properties of canine skin have been found to be more similar to human skin than the properties of pig skin (Bartell and Mustoe, 1989). The expense and size of the model has limited its use when other models (rabbit, rat) can be substituted adequately.

### **Perfusion measurement**

Direct observation of a flap is the most common method of assessing flap viability in clinical situations. Findings such as flap color, temperature, capillary refill, and bleeding at the distal edge give gross approximation of flap perfusion. Greater reliability is needed in clinical flaps having questionable viability and in the laboratory.

Perfusion measurements are used in research to (1) quantify the effect of a particular agent on the blood flow to a flap, (2) obtain a baseline measurement to ensure that experimental and control flaps have an equivalent blood flow, and (3) predict the survival of a flap. Clinical uses of perfusion measurement have included monitoring the blood flow to a flap postoperatively and predicting flap viability at the time of surgery. Some of the techniques of perfusion measurement in skin flap research that are new or in frequent use are reviewed.

### ***Microspheres***

Microspheres are thought to be the most accurate means of estimating blood flow (Myers, 1986). The technique depends on three principles. First, the microspheres must be distributed to the tissues in direct proportion to their blood flow. For this to happen they must be well mixed and rheologically similar to red blood cells. Second, the microspheres must be trapped in the capillary bed in the first circulation. Finally, the systemic hemodynamics must not be affected by the embolization of the capillary bed (Pang et al, 1984).

Microspheres are polystyrene beads of uniform size with isotopes placed inside. For measurement of capillary perfusion the beads are typically 15 microm in size to allow trapping in the capillary beds but passage through A-V shunts. Larger microspheres (50 microm) can be used if trapping in the A-V shunts is desired. Microspheres are injected into the left ventricle, where they are mixed before being expelled with the blood and trapped in the tissue capillaries. The ratio of the blood flow in a specific tissue to the cardiac output equals the ratio of the number of microspheres trapped in that tissue to the total number of spheres injected. A blood sample is drawn at the time of microsphere injection to serve as a reference for calculating cardiac output and blood flow to the tissue being investigated (Pang et al, 1984).

The microsphere technique was found to be linearly correlated with blood flow to the skin (Pang et al, 1984). When skin blood flow was low (approximately 0.03 mL/min), the repeatability of the technique was hindered. A blood flow this low is rarely seen in acute random flaps and would occur only when arterial spasm is at a maximum in the early postoperative period. By using different sets of microspheres, capillary blood flow can be measured simultaneously and consecutively to skin, muscle, and bone. A major disadvantage preventing its clinical use is the need to sample the tissue at the end of the experiment.

### *Fluorescein*

Many vital dyes are available, including bromphenol, disulphine, patent blue, vicodan, and xylenol orange, but fluorescein is used most often. Sodium fluorescein dye ( $C_{20}H_{10}Na_2O_5$ ; molecular weight 376.3) is nontoxic at pharmacological doses of 10 to 15 mg/kg (Pang et al, 1986a). LD-50 is 1000 mg/kg in laboratory animals. When exposed to ultraviolet light (< 510 nanom), the dye will emit a yellow-green fluorescence. After intravenous injection the fluorescein moves quickly from the intravascular compartment to the extracellular space without penetrating the cell membranes. Staining occurs in tissues with a nutrient blood flow. Fluorescence can be detected visually with a Wood's light, photographically with the appropriate filters, or with a dermofluorometer.

The visual fluorescein test is performed with a Wood's light, and the length of fluorescein staining is observed. With fluorescein photography a blue filter is placed over the flash and a yellow filter is placed over the lens. Both techniques require a relatively large dose of fluorescein (15 to 30 mg/kg). This dose can take 12 to 18 hours to clear, which limits how often the test can be performed. Both techniques are more difficult to perform in highly pigmented skin.

Lower doses of fluorescein (1.5 mg/kg) can be used with a fiberoptic dermofluorometer. The dermofluorometer uses a fiberoptic cable to carry the ultraviolet light to the skin and transmit the induced fluorescence to a photodetector. A numerical output is generated, which can be read off the machine. For each estimation of blood flow, the skin fluorescence before and after fluorescein injection is measured. The rise in fluorescence of the skin under investigation and in a reference area are compared, and a dye fluorescence index (DFI) is calculated. By quantifying the fluorescence and lowering the fluorescein dose, blood flow can be examined at more frequent intervals.

Areas in a skin flap with a rise in fluorescence that is approximately 30% of an area of normal skin (DFI = 30) would be expected to survive (Cummings et al, 1984; Sloan and Sasaki, 1985). This technique has been used to determine the optimal time for pedicle division of a regional flap (Gatti et al, 1984). An underestimation of actual skin flap survival with fluorescein has been noted when fluorescein is given early in the postoperative period. At 1 hour after creating a skin flap the visual fluorescein test was found to underestimate skin flap survival by approximately 20% (Pang et al, 1986a). At 18 to 24 hours after surgery the flap survival was highly correlated with actual survival (Pang et al, 1986a; Sloan and Sasaki, 1985). The underestimation of skin flap survival was thought to be due to postoperative arteriospasm in the distal portion of the flap (Pang et al, 1986a). Taking the changes in flap blood flow into account, Thomson and Kerrigan (1989) found a DFI of 7 was associated with flap survival when measured in the first 2 hours after surgery. A DFI of 27 was needed to ensure survival when measured 5 hours after flap elevation.

Fluorescein uptake used as an indication of blood flow limits the measurement to intermittent readings. Fiberoptic fluorometry can also be used to monitor fluorescein washout or clearance (Denneny et al, 1986). Studies were done with arterial occlusion, venous occlusion, or pedicle occlusion for 30 minutes. With arterial occlusion a dramatic decrease in fluorescein elimination occurred and control levels returned with release of the clamp. With venous occlusion no fluorescein elimination occurred and partial occlusion led to prolonged elimination. The study concluded that by measuring fluorescein elimination, tissue perfusion could be monitored continuously.

### *Laser doppler*

With the laser doppler a 2-mW helium-neon laser is used to produce a uniform light with a wavelength in air of 632.8 nanom. A fiberoptic cable is used to carry the light to the skin surface and to transmit the backscattered light to a photo-detector. Three different types of measurement can be obtained with a laser doppler. A laser doppler flow (LDF) signal is generated by measuring the movement of red blood cells. The doppler effect results in backscattered light from the surface of a stationary tissue plane having a different wavelength than light backscattered from a moving object (in this case a red blood cell). The number and average velocity of red blood cells determine the LDF value. A second measurement is laser photometry (LP). This signal is generated by the total intensity of backscattered light. At this wavelength light is mainly absorbed in the skin by the hemoglobin in red blood cells, so the LP value is inversely proportional to the blood volume of the tissue studied. In newer versions of the laser doppler the velocity of the red blood cells can be calculated (Phillips et al, 1989).

Svensson et al (1985) used the LDF and LP outputs of the laser doppler to monitor free flaps and found that arterial and venous occlusion could be differentiated. With arterial or venous occlusion a dramatic decrease in the LDF occurred because of a lack of flow through the tissue. LP was noted to be unchanged or slightly increased with arterial occlusion, but dramatically decreased with venous occlusion, suggesting tissue engorgement. Phillips et al (1989) analyzed the LDF, blood volume, and velocity outputs of the laser doppler and found differences between arterial and venous occlusion in buttock island flaps raised in pigs. In this experiment the laser doppler detected decreased blood flow as early as 10 minutes after clamping the pedicle and could differentiate whether there was arterial or venous occlusion. At 60 minutes after arterial occlusion a 70% decrease in LDF, an 85% decrease

in volume, but only a 10% decrease in velocity were observed. Sixty minutes after a venous occlusion a moderate decrease in LDF (70%) and velocity (50%), but only a small decrease in volume (15%) were detected.

The quality of blood flow estimation with the laser doppler has had a mixed review. Marks et al (1984) felt that the laser doppler, like fluorescein, becomes more accurate at 24 hours after flap elevation. Liu et al (1986) felt that the laser doppler was more likely to reflect nonnutritive blood flow in the immediate period after flap elevation. Sloan and Sasaki (1985) found the laser doppler to have an increased variability and had difficulty reproducing results. Reports of blood flow in nonperfused tissue have also been published (Fischer et al, 1985; Marks et al, 1984; Sloan and Sasaki, 1985). For this reason percentage change rather than absolute values are often followed when using the laser doppler clinically.

Heden et al (1986) found the laser doppler to be as accurate as fluorescein in the radial dorsal flap if certain techniques were followed. This included immobilization of the skin to probe interface and monitoring a single site. The laser doppler has been used to monitor myocutaneous flaps (Cummings et al, 1984) and free flaps in humans. The laser doppler has the advantage of being relatively simple to use and is noninvasive and thus is an attractive option for continuous monitoring of revascularized tissue (Silverman et al, 1985).

### *Metabolic monitoring*

Glinz and Clodius (1972) evaluated pH measurements in the subcutaneous tissue of pig pedicle flaps and found pH to be a reliable indicator of tissue necrosis. They found that tissues with a pH more than 0.35 units lower than adjacent normal tissue did not survive. Dickson and Sharpe (1985) studied pH changes in rat epigastric island flaps by placing a pH probe into the middle of the flap. They found a measurable fall in pH within a minute of clamping the pedicle. Arterial occlusion led to a faster pH fall to lower values than venous occlusion. In pig rectus abdominis flaps, pH measurement had similar results (Warner et al, 1989). The pH changes in their study were nearly identical for the subcutaneous and muscular layers.

Mahone and Lista (1988) used a  $pO_2$  probe to monitor rabbit epigastric flaps. The measurement is based on the reduction of oxygen across an electrode pair. The  $pO_2$  of the surrounding tissue is proportional to the current produced between the anode-cathode gap. In this study, arterial and venous occlusion could not be differentiated. Occlusion of the entire pedicle could be detected with an oxygen challenge test in which 100% oxygen was administered. If the pedicle was intact, measured  $pO_2$  increased three to four fold. This increase was not present when the pedicle was occluded.

Temperature monitoring of flaps has been criticized for having a slow response time and a small response (Warner et al, 1989). Monitoring of surface temperature is easy to obtain and requires simple equipment. Temperature probes can provide a continuous estimate of flap perfusion. Skin temperature is related to blood flow but not always in a predictable or reliable fashion. The temperature-blood flow relationship is influenced by core temperature, air temperature, humidity, light, and vasomotor responses. In the laboratory, Sloan and Sasaki (1985) found good correlation between temperature and survival, but noted that pedicle occlusion will be detected earlier by administration of cutaneous oxygen and fluorescein.

## *Clearance*

The rate of removal of a particular substance can be used to estimate blood flow. Xenon, iodide, and sodium isotopes have been used in this regard. Hydrogen gas clearance and technetium are two techniques recently discussed in the literature. The use of fluorescein clearance has already been described.

Measurement of hydrogen gas clearances uses electrodes placed into the dermis through a needle. A current is applied to one wire and the nearby hydrogen is ionized. The resulting microelectric current is measured by a second wire, and a clearance curve indicating the decline of hydrogen concentration is generated. Blood flow approximates the slope of the hydrogen clearance curve plotted against time. Koshu et al (1982) used a higher initial concentration of hydrogen to reduce the variability of the readings with hydrogen gas clearance. Suzuki et al (1985) found no influence on flap survival by the needle injections and the clearance of hydrogen correlated well with survival length and fluorescein staining. An advantage of the technique is the lack of a radioactive substance. The authors did not that there are several technical points that are important in obtaining an accurate and reliable measurement. These included the method of needle insertion and the temperature of the room and the skin.

Technetium 99 pertechnetate clearance was used by Waterhouse et al (1986) to estimate blood flow. The isotope was injected intradermally and 240 readings were taken over a period of 40 minutes. Decreased clearance was associated with a decreased blood flow. The measured clearance rate was divided into a fast component occurring at 2 to 8 minutes postinjection and a slow component during the 28- to 40-minute readings. Young and Howell (1980) believe that isotope clearance is related to the depth of injection in pig skin. The fast component was related to clearance from the superficial papillary dermis, which comprises 90% of skin blood flow and is involved with thermoregulation. The other 10% was found mostly in the deeper reticular dermis and was thought to comprise the slow component. In the study by Waterhouse et al (1986) a single clearance curve, implying loss of thermoregulatory blood flow, was seen in ischemic skin. Split-thickness skin grafts were also found to have a single clearance curve, suggesting that such grafts are revascularized as a single functional unit. This technique, however, was not felt to be useful in monitoring clinical flaps in the postoperative period.

## **Measurement of interstitial changes**

Dynamic interstitial tissue compliance (change in volume or pressure) has been measured by injecting a small amount of fluid into a tissue and measuring the change in pressure (Odland and Cohen, 1988). The measurement reflects interstitial tissue pressure, which increases with ischemia and inflammation. Tissue pressure was found to be increased at all sites after flap elevation compared to normal skin in the rat dorsal flap model. Distal location and increased time after flap elevation up to 18 hours was associated with further increases in interstitial pressure. Increased interstitial pressure is a potential factor in the no-reflow phenomenon (Rosen et al, 1985) and in critical flaps limiting capillary nutrient blood flow and diffusion of nutrients into the interstitial space.

Magnetic resonance imaging may be another way to investigate changes in the intercellular space after flap elevation. Interruption of the blood supply to the canine gracilis muscle flap resulted in a tendency for increased spin-spin relaxation time ( $T_2$ ) but the sample was too small to be statistically significant (Greenberg et al, 1987).

### **Attempts to Alter Skin-Flap Viability**

Kerrigan (1983) outlined extrinsic and intrinsic causes of skin-flap failure. Extrinsic reasons for flap necrosis are those not resulting from the design of the raised flap. Examples include systemic hypotension, infection, and pedicle compression. Often, these factors can be overcome in the clinical situation. The primary intrinsic factor affecting flap survival is inadequate blood flow. Numerous experimental attempts have been made to influence flap microcirculation and/or decrease the deleterious effects of inadequate flap blood flow. The most successful has been flap delay. Attempts to improve flap blood flow and cutaneous tolerance of ischemia have been less successful but continue to be active areas of research.

### **Delay**

Four facts are accepted about the delay phenomenon. First, it requires surgical trauma; second, a large percentage of the neurovascular supply to the flap must be eliminated; third, delay results in increased flap survival at the time of tissue transfer; and fourth, the beneficial effects can last up to 6 weeks in the human (Pearl, 1984). To explain this phenomenon, three theories regarding the mechanism of delay have been developed: (1) delay improves the blood flow, (2) delay conditions the tissue to ischemia (McFarlane et al, 1965b), and (3) delay closes arteriovenous shunts (Reinsch, 1974). The most recent research supports a mechanism resulting in increased circulation to the flap, but how this occurs remains to be proved.

Using microspheres Pang et al (1986b) found the percentage of arteriovenous shunt flow to be similar in delayed and acute flaps. The increased blood flow in delayed flaps was caused by an increase in total blood flow. The addition of systemic norepinephrine decreases the blood flow in delayed flaps to the level seen in acute flaps. The increased survival in the delayed flaps was thought to be caused by a decrease in vasoconstriction in the distal portion of the flap.

Flaps delayed as little as 24 hours survive to a greater length (Sasaki and Pang, 1981) and can tolerate longer periods of ischemia (Weinberg et al, 1985, 1985). Using the microsphere technique, distal perfusion in the pig flank flap was found to increase with a delay up to 4 days. No further increase in perfusion was seen with continued delay up to 14 days (Pang et al, 1986c). The early increase in blood flow was thought to occur too early to be caused by angiogenesis and a bipedicle flap design limited the effect of hypoxia as a stimulus. Pang et al (1986c) theorized that a modulation of the vasoactivity of the small arteries allowed delivery of more blood to the distal portion of the flap. This modulation could occur by release of vasoconstrictive substances (norepinephrine, thromboxane, and serotonin) during elevation of the bipedicle flap. Necrosis is not seen because the bipedicle flap has an adequate blood supply. Degeneration release of norepinephrine occurs soon after flap elevation and norepinephrine stores are largely depleted in the first 24 to 48 hours (Jurell, 1986; Palmer, 1970). In bipedicle flaps, Cutting et al (1982) found that the catecholamine level started to rise 4 days after flap construction, whereas others (Jurell, 1986; Palmer, 1970)

found the catecholamine levels to be depressed over a greater period. After depletion of the catecholamines a relative state of sympathectomy develops. Complete sympathetic denervation is unlikely in pedicled flaps because of the presence of sympathetic fibers in the periarteriolar tissue and between the media and adventitia of the vascular wall (Marshall, 1976; Somlyo and Somlyo, 1970). Because of the catecholamine depletion, conversion of the delayed flap to a single pedicle at this time is not accompanied by the same degree of vasoconstriction (Pang et al, 1986b).

Early after elevation the vasculature to the flap has an increased sensitivity to the effects of adrenergic drugs (Pearl, 1981). Intravenous norepinephrine was shown to result in decreased blood flow to a myocutaneous flap in the porcine model despite increased blood flow to control skin (Moore et al, 1986). This represented a hypersensitivity to exogenous norepinephrine in this model. The investigators were also able to demonstrate a blunting of the norepinephrine-induced pressor effects by treatment with phenoxybenzamine, an alpha-adrenergic blocking agent. The hypersensitivity to norepinephrine was seen at 2 and 5 days after flap elevation, which is similar to the period of 1 to 7 days after flap elevation when decreased tissue norepinephrine was found by Cutting et al (1982). This recovery from the hyperadrenergic state appears to play a role in the delay phenomenon.

Development of vascular collaterals and reorientation of the major vascular channels is another mechanism for increasing blood flow to the distal portion of the single pedicle flap (Cutting et al, 1981; Guba, 1979). Using the rat dorsal flap model, Suzuki et al (1988) found the delay effect to be greater in narrow flaps as opposed to wide flaps. The longitudinal channeling was thought to be greater in the narrow flaps because more of the transverse vessels were cut. Longitudinal flow is also enhanced by vasodilating substances released by inflammation and mild ischemia (Suzuki et al, 1988). Pang et al (1986b) believed that the depletion of vasoconstricting substances played a role in the early stage of delay whereas locally released vasodilating substances were involved in the later stages.

## **Increase blood supply**

### *Vasodilators*

**Indirect.** The intense vasoconstriction associated with release of norepinephrine in the early period after flap elevation would seem to hinder flap survival. As discussed above, one of the benefits of flap delay seems to be depletion of norepinephrine before the flap to be transferred is created. If this vasoconstriction could be blocked or reversed, the duration and severity of distal flap ischemia should be decreased. The result would be increased flap survival without the need for delay.

Alpha-adrenergic blocking agents are directed against the catecholamine-induced vasoconstriction seen after flap elevation. Using the rat model, phenoxybenzamine resulted in improving flap survival in some studies (Finseth and Zimmerman, 1979; Myers and Cherry, 1968; Wexler et al, 1975). Phenoxybenzamine and phentolamine ointments applied topically were also found to be effective in increasing flap survival in the rat model (Goshen et al, 1985). Other investigators have been unable to reproduce beneficial effects in the rabbit or pig (Kerrigan and Daniel, 1982a; Myers, 1975). Depletion of norepinephrine stores before flap elevation with reserpine (Cutting et al, 1978; Jurrell and Jonsson, 1976; Kennedy et al, 1979;

Kerrigan and Daniel, 1982a) and guanethidine (Aarts, 1980; Finseth and Adelberg, 1978; Hannigton-Kiff, 1974) has also met with mixed results and systemic toxicity.

**Direct.** Direct vasodilators such as histamine, hydralazine, and topical dimethylsulfoxide have showed both beneficial effects and no effects on skin-flap survival (Kerrigan and Daniel, 1982a). Isoxsuprine is a phenylethylamine derivative of epinephrine having alpha-adrenergic receptor antagonist and beta-adrenergic receptor agonistic properties resulting in relaxation of vascular smooth muscle. In high doses it can decrease viscosity and inhibit platelet aggregation. This combination of actions and early experimental studies (Finseth and Adelberg, 1978; Finseth and Zimmerman, 1979) created optimism and a flurry of research on the effects of using isoxsuprine. Subsequent studies have shown minimal or no beneficial effects of isoxsuprine on skin-flap survival (Kerrigan and Daniel, 1984; Neligan et al, 1985; Pang et al, 1985; Wray and Young, 1984).

Using the microsphere technique, isoxsuprine was found to increase blood flow in the area of the dominant artery in porcine myocutaneous and arterial flaps. No increase in blood flow was seen in the distal random portion of the flaps or in flap survival (Neligan et al, 1985; Pang et al, 1985). Similar results were obtained with diazoxide, a non-diuretic thiazide and a direct dilator of arterial smooth muscle (Pang et al, 1985). The smaller vessels in the distal random portion of a flap were theorized to have a different sensitivity to vasodilator drugs than muscular or axial arteries. Manipulation of these distal vascular channels appears to be critical in increasing flap survival.

Calcitonin gene-related peptide (CGRP) is a bioactive neuropeptide found in primary sensory neurons. A potent vasodilator, it is thought to stimulate smooth muscle relaxation by an endothelial-dependent mechanism. CGRP has been shown to improve blood flow in the rat epigastric flap (Knight et al, 1988), to improve survival in ischemic skin flaps in rats (Kjartansson et al, 1987a), and to delay onset of the no-reflow phenomenon (Westin and Heden, 1988). Pretreatment with capsaicin, which depletes neuropeptides from primary sensory neurons, results in a decreased survival of dorsal flaps in the rat (Kjartansson et al, 1987b). These findings suggest a potential role for primary sensory neurons in cutaneous vascular control and flap survival.

Acupuncture has also resulted in increased flap survival in the rat dorsal flap model (Jansen et al, 1989a). In the same model, electro-acupuncture increased blood flow in the same was as injection of CGRP (Jansen et al, 1989b). This supports the theory of a mechanism involving release of vasodilatory substances from sensory neurons causing the improved survival after acupuncture, but further study is needed.

Topical application of vasodilating drugs directly to the skin-flap surface has had mixed results. Topical nitroglycerin (NTG) was found to increase survival in the rat abdominal flap and the porcine axial flank flap (Rohrich et al, 1984). NTG acts as a vasodilator with more potent venodilator than arteriodilator effects. Its action on veins was felt to contribute to the increased flap survival. When used on the cranially based dorsal rat flap, however, NTG did not result in improved survival (Nichter et al, 1985). On the other hand, intraperitoneal dimethyl sulfoxide significantly increased flap survival in the rat abdominal flap (Haller et al, 1987) despite its inconsistent record as a topical agent (Arturson and Khanna, 1970; Myers and Donovan, 1973).

Calcium channel blockers are potent vasodilators that can potentially reduce necrosis by keeping calcium from entering ischemic cells. Because they are already used clinically for other disorders, they were thought to be promising for salvaging a critically ischemic flap (Myers, 1986). Nifedipine increased survival in rat dorsal flaps (Hira et al, 1990) but not in porcine dorsal random flank flap (Miller et al, 1985). The lack of beneficial effect in the pig model may have been caused by a decrease in systemic blood pressure. In the rat dorsal flap model, verapamil given by intraperitoneal injection also failed to increase flap survival over a control (Nichter and Sobieski, 1988). The failure of calcium channel blockers or other direct vasodilators to increase flap survival reproducibly indicates that mechanisms other than direct arterial dilatation are important in survival of the ischemic flap.

### Alter rheology

In a homogeneous fluid that exhibits equal shear stress at different rates of shear, flow (Q) in a vessel can be approximated by the Poiseuille equation:

$$Q = (\text{delta}P * r^4 * \pi) / l * 8 * n$$

where *deltaP* equals pressure gradient,  $r^4$  equals the fourth power of the vessel radius, *l* equals vessel length, and *n* equals viscosity (Guyton, 1976). Although blood is a non-Newtonian fluid, the qualitative relationships in the equation remain applicable. In larger vessels of the circulation vessel radius is a dominant factor, but in the capillary microcirculation viscosity is more important. By decreasing the viscosity of blood it may be possible to increase flow to the distal random portion of the acutely raised flap and improve flap survival. Viscosity is influenced by the hematocrit, serum proteins, temperature, red blood cell deformability and aggregation, as well as other factors (Roth et al, 1988). Each of these factors can be potentially manipulated with a resultant change in viscosity.

Hemodilution has been shown to decrease viscosity and have a beneficial effect on flap survival (Earle et al, 1974; Neilsen and Parkin, 1976; Ramasastry et al, 1985). Reducing blood viscosity by protein depletion also results in increased flap survival in rats (Ruberg and Falcone, 1978). Dextran solutions also result in decreased blood viscosity but a reproducible improvement in survival has not been attained (Goulain, 1967; Grabb and O'Neal, 1966).

Pentoxifylline is a hemorrheologic agent used in the treatment of intermittent claudication. Chemically, it is a tri-substituted xanthine related to caffeine and theophylline. Pentoxifylline increases intracellular adenine triphosphate levels in red blood cells, which results in increased red blood cell deformability (Ehrly, 1976). Other effects include decreasing serum fibrinogen and platelet aggregability (Roth et al, 1988).

A number of experiments have examined the effects of pentoxifylline on skin-flap survival. When given 7 to 10 days before flap elevation, pentoxifylline has resulted in increased flap survival in porcine dorsal flank flaps (Yessenow and Maves, 1989) and the rat dorsal flap (Roth et al, 1988). The increased survival was associated with a decrease in viscosity (Roth et al, 1988). Pentoxifylline needs to be administered for 2 to 4 weeks in order to achieve the desired effect (Yessenow and Maves, 1989).

Some studies have found increased survival with 24 hours or less of preoperative pentoxifylline (Hauben and Aijlstra, 1984; Monteiro et al, 1986; Nemiroff, 1988). Despite a 50% increase in surviving length in treated flaps, no increase in distal blood flow could be detected using tagged red blood cells (Monteiro et al, 1986). Viscosity measurements also failed to show a difference from control (Roth et al, 1988). Direct measurements of red blood cell deformability after 24 hours of pentoxifylline treatment may provide an explanation for the increase in survival. Beneficial effects with limited preoperative dosing of pentoxifylline have not been uniform. No improvement in flap survival was seen in the rabbit caudally based dorsal flap (Chu and Deshmukh, 1989) and the porcine dorsal flank flap (Hodgson et al, 1987).

Fluosol-DA is a whole-blood substitute with low viscosity (particle size = 0.1 microm) and a high oxygen-carrying capacity (Chowdary et al, 1987b). When used alone Fluosol-DA (20%) failed to increase flap survival (Ramasastry et al, 1985). When combined with a high-oxygen environment, a beneficial effect on flap survival has not been consistent in the rat model (Chowdary et al, 1987b; Ramasastry et al, 1985). Another fluorocarbon, oxypherol-ET, was found to increase survival in porcine flank flaps (Yessenow and Maves, 1988) but this has yet to be confirmed in other laboratories.

### *Inflammation*

The surgical trauma associated with an acutely raised or delayed flap results in an inflammatory response. This response results in a local increase in blood flow, which could improve flap survival. Recent investigations have attempted to improve flap survival with different methods of creating an inflammatory response as well as to determine the mechanism by which inflammation produces a beneficial effect.

Preoperative application of croton oil, which produces a superficial burn similar to a chemical peel, resulted in increased survival length in the dorsal rat flap (Liston, 1984). Increased survival was also seen after injecting 0.2% formic acid below the pannus in the rat dorsum (Lawrence et al, 1984). In contrast, application of a chemical peel to porcine dorsal flank flaps 48 hours before elevation did not result in improved survival length over control flaps (Gaughan et al, 1986). The untreated flaps in this study had a chemical peel applied to flaps 2 cm away on either side and may have benefited from the nearby inflammation. A similar mechanism was thought to be responsible for increased blood flow in skin flaps raised adjacent to previously delayed flaps (Jonsson et al, 1988).

Low-power laser burns to the skin applied daily for 5 days either preoperatively or postoperatively resulted in increased flap survival in the dorsal rat flap (Kami et al, 1985). Examination of laser burn sites in unoperated skin showed increased blood flow measured by hydrogen clearance. Histologic exam of the burn sites showed hypovascular areas at the burn sites 1 hour after irradiation but increased blood vessel proliferation 2 days after irradiation. These studies demonstrate that the inflammatory response can be a stimulus for delay without sympathectomy or vascular division.

Cyclooxygenase inhibitors, such as indomethacin, and ibuprofen have been shown to increase skin-flap viability (Robson et al, 1979; Sasaki and Pang, 1981). Glucocorticoids that inhibit phospholipase A<sub>2</sub> activity have increased flap survival in some studies (Kristensen et

al, 1978; Mendelson and Woods, 1978; Mes, 1980). In the porcine model however, methylprednisolone was found to have no effect on survival or blood flow when given preoperatively or early in the postoperative period (Nakatsuka et al, 1985). Ibuprofen was also demonstrated to result in prolonged tolerance of ischemia (Douglas et al, 1987).

Studies have also been performed using prostaglandins as experimental agents. Administration of prostacyclin has been shown to have a beneficial effect on flap survival in rate (Emerson and Sykes, 1981; Sasaki and Pang, 1981). Dilatation of the arterial pedicle and an increase in the total blood flow was seen in a nonischemic rabbit epigastric flap after local injection of prostacyclin (Knight et al, 1985). Low doses of alpha-cyclodextrin clathrate (a stable PGE<sub>1</sub>) had a beneficial effect on blood flow measured by hydrogen gas clearance and survival in a rabbit dorsal flap. At higher doses a resultant hypotension seemed to prevent an increase in blood flow to the flap (Suzuki et al, 1987). Low-dose PGI<sub>2</sub> has also been shown to enhance flap survival in the pig (Reus et al, 1984). In the rat model a topically effective analogue of PGE<sub>2</sub> was found to improve flap tolerance of 10 hours of pedicle occlusion (Silverman et al, 1989).

Blocking TxA<sub>2</sub> synthesis has had mixed results. TxA<sub>2</sub> inhibition with dazmegrel, which should increase the effect of prostacyclin, resulted in no effect in the rat dorsal flap (Kay and Green, 1986). More recent studies have found inhibition of thromboxane synthetase to be successful in increasing ischemic flap viability (Ono et al, 1990) and tolerance of skin flaps to secondary ischemia (Mellow et al, 1990).

### *Other*

A number of drugs with multiple effects that should alter the blood flow to the flap have been used with varying results. Ancrod, a defibrinogenating enzyme from the pit viper, has a selective affinity for fibrinogen and stimulates release of plasminogen activator with a resultant decrease in viscosity. The drug also causes increased endothelial generation of prostacyclin. When given postoperatively for 7 days, ancrod was unable to increase the viable surface area of porcine myocutaneous flaps (Moore and Cummings, 1988).

Chlorpromazine's effects include alpha-adrenergic blockade, stabilization of cell membranes, serotonin antagonism, metabolic depression, and cooling. All these effects should be beneficial to the ischemic flap but the results from experimental studies have been mixed. No improvement in survival of the rat dorsal flap was seen when chlorpromazine was given 30 minutes preoperatively and up to 7 days postoperatively. There was a significant improvement, however, when the postoperative treatment was continued for 14 days. The mechanism for this delayed beneficial effect is unknown (Bibi et al, 1986). Angel et al (1989) found chlorpromazine given preoperatively and 7 days postoperatively resulted in increased survival of dorsal rat skin flaps. Of chlorpromazine's effects, the increase in blood flow was believed to be more important than membrane stabilization. In contrast, chlorpromazine given preoperatively and 10 days postoperatively had no beneficial effect on flap survival in rats (Hoft et al, 1990).

Application of a semipermeable membrane to the flap surface resulted in increased survival in the rat dorsal flap (Nichter et al, 1985). The improvement seen may be due to preservation of a moist environment and reduced depth of tissue loss (McGrath, 1981). In a

study by Kaufman et al (1985) topical ointments were a hindrance to flap survival. Pressure dressings, however, resulted in increased survival due to improved contact between the flap and the recipient bed.

The length of time a flap is dependent on its pedicle is determined by the rate of capillary ingrowth into the transferred tissue. Separation of porcine myocutaneous flaps from the recipient bed with silastic sheeting resulted in a prolongation of the time needed for revascularization (Millican and Poole, 1985b). In the same model, revascularization of muscle flaps required twice as much time as myocutaneous flaps demonstrating the importance of the dermal and subdermal vascular plexus. A potentially useful manipulation of neovascularization was demonstrated by Hom et al (1988). Increased flap survival and vascularity was seen when an endothelial growth supplement was applied in a sustained-release fashion.

Tissue expansion has been demonstrated to increase the size of the transferred flap in experimental animals and man. Examination of expanded skin in the guinea pig has shown an increase in the thickness (Austad et al, 1982) and mitotic activity (Francies and Marks, 1977) of the epidermal layer, indicating epidermal proliferation. Blood flow in expanded tissue is greater than in skin overlying a noninflated expander 1 hour after creation of a pedicled flap in the porcine model (Marks et al, 1986). The increased blood flow to expanded skin when compared to delay seems to be short-lived (Goding et al, 1988; Ricciardeli et al, 1989). Apart from the acute changes seen with expander manipulation, flap viability and blood flow in expanded skin appears to be similar to that seen in delayed flaps (Sasaki and Pang, 1984).

An interesting manipulation to increase survival of thin axial pattern skin flaps was proposed by Morrison et al (1990). In their study, the femoral artery and vein was implanted into the subdermal layer of skin in the rabbit model. After 8 to 12 weeks sufficient neovascularization had occurred to allow creation of a large skin flap based on the transferred pedicle. If confirmed in other laboratories, this technique may allow greater flexibility in the design of axial pattern flaps.

## **Prolonged viability**

### ***Protection against harmful agents***

The production of free radicals with reperfusion and the return of molecular oxygen to ischaemic tissue have been a recent focus of experiments attempting to improve flap survival. This research has focused on decreasing the production of free radicals and using agents that remove free radicals (free radical scavengers) from the immediate environment.

Preoperative administration of allopurinol (a xanthine oxidase inhibitor) prevents the increased xanthine oxidase activity seen with acute flap elevation (Im et al, 1984). Improved survival of dorsal rat flaps has been accomplished with allopurinol when given at high doses (Angel et al, 1987; Pokorny et al, 1989), with lower doses having no effect (Angel et al, 1987). The high doses required have led to concern about the use of allopurinol to increase flap survival in humans.

A number of free radical scavengers are available to protect the tissues from destruction by free radicals. Superoxide dismutase (SOD), an intracellular free radical scavenger, catalyzes the conversion of superoxide to hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and molecular oxygen. When given systemically, SOD is an effective scavenger of the superoxide radical regardless of its source (McCord, 1986). SOD treatment has resulted in improved flap survival (Freeman et al, 1990; Pokorny et al, 1989; Zimmerman et al, 1987) and increased tolerance to ischemia in rat abdominal flaps (Marzella et al, 1988; Sagi et al, 1986). The beneficial effects of SOD appear to be partially suppressed when given in physiologic solutions, which may raise the pH of the ischemic tissue (Sagi et al, 1990). Administration of a copper chelator decreased flap survival in the rat abdominal flap model by deactivating endogenous SOD. This effect could be overcome by administration of relatively large doses of exogenous SOD (Freeman et al, 1990).

Prolongation of the circulating half-life of SOD (normally 6 minutes) up to 30 hours (Boccu et al, 1982) can be accomplished by the attachment of polyethylene glycol to the molecule (PEG-SOD). Use of PEG-SOD improved flap survival of rat abdominal flaps compared to SOD and controls (Huang et al, 1987). Improved flap survival has also been demonstrated with a number of other naturally occurring compounds with free radical scavenging properties. These include deferoxamine (Angel et al, 1986a), vitamin E, vitamin A, vitamin C, glutathione (Hayden et al, 1987), various amino acids (Paniello et al, 1988) and amino-acid derivatives (Kim et al, 1990).

The hydrogen peroxide formed by the dismutation of superoxide is not particularly harmful. In the presence of chelated metal complexes, however, hydroxyl radicals (OH<sup>-</sup>) are formed through a Fenton or Haber-Weiss reaction (Del Mastro, 1980). The hydroxyl radical is much more reactive and may be responsible for much of the damage inflicted by oxygen-free radicals (Southorn and Powis, 1988). Hydroxyl radical activity in skin-flap necrosis is implicated by the ability of dimethyl thiourea, a specific hydroxyl radical scavenger, in improving survival in rat abdominal flaps (Hayden et al, 1988).

That iron acts as a catalyst producing free radicals in hematoma-induced flap necrosis is supported by Angel et al (1986b). In their experiment, blood instilled under ischemic rat dorsal flaps resulted in increased necrosis as compared to plasma or controls. Addition of deferoxamine, a specific iron chelator, to the instilled blood lessened the necrosis, whereas adding ferrous sulfate to instilled plasma increased the necrosis. Malonyldialdehyde, a product of free-radical-catalyzed lipid peroxidation, was increased in necrotic flaps overlying a hematoma, further implicating free radicals in this process.

### *Increased tolerance of ischemia*

**Increased oxygenation.** Improvement in flap survival with hyperbaric oxygen treatment has been documented in the rat model (Kernahan et al, 1965; Perrins, 1975). Hyperbaric oxygen treatment, however, increases blood oxygen-carrying capacity by 20%, at the most (Kernahan et al, 1965). A greater effect of hyperbaric oxygen treatment may be to increase oxygen diffusion from surrounding perfused tissue to the ischaemic portion of the flap (Cutting, 1986). Increased flap survival occurs with treatments using 21% hyperbaric oxygen, implying that increased oxygen could be delivered with an increase in pressure alone (Tan et al, 1984).

An 8-hour treatment of hyperbaric oxygen improved survival of rad dorsal flaps when given 48 hours before or 4 hours after flap elevation. No effect was seen with hyperbaric oxygen given 24 hours before or 48 hours after surgery (Nemiroff et al, 1985). This is consistent with earlier findings that a treatment delay of 24 hours or more after surgery results in little benefit from hyperbaric oxygen (Jurell and Kaijser, 1973). The effectiveness of hyperbaric oxygen given 48 hours preoperatively was thought to be the result of allowing enough time for increased fibroblastic activity and capillary ingrowth to occur. An experiment using a porcine model was less successful in improving flap survival. Hyperbaric oxygen given within 4 hours after surgery showed no difference in survival of flank, axial buttock, and random buttock flaps in the pig (Caffe and Gallagher, 1988).

Nemiroff (1988) studied hyperbaric oxygen and pentoxifylline in the rat dorsal flap model. Both agents individually improved flap survival over a control group but had a synergistic effect when used in combination. This effect was felt to be a result of their separate but complementary mechanisms of action.

**Metabolic manipulation.** Decreasing the metabolic requirements or increasing the metabolic reserves of skin are additional strategies for increasing flap survival. These approaches are based on the concept that flap necrosis occurs when tissue metabolic demand is greater than blood supply. Decreasing temperature is an effective way to reduce metabolic activity. Local hypothermia results in a dramatic decrease in blood flow to porcine myocutaneous flaps without hindering ultimate survival (Goding et al, 1991). A delay in necrotic changes has been noted earlier (Kiehn and Desprez, 1960). In both studies improvement in flap survival was not seen.

Administration of adenosine triphosphate-magnesium chloride complex was used to increase survival in the rat abdominal flap (Zimmerman et al, 1987). The metabolic support provided by this compound was felt to delay the onset of irreversible cell damage. Difluoromethylornithine (DFMO) was found to increase survival of abdominal flaps in rats (Perona et al, 1990). DFMO appears to reduce metabolic demand by inhibiting the synthesis of polyamines, which are required for protein synthesis and cellular proliferation.

### **Impaired flaps**

Smoking tobacco is associated with an increased chance of flap necrosis in face-lift operations (Rees et al, 1984). Exposure to tobacco smoke resulted in increased flap necrosis of dorsal flaps in rats (Nolan et al, 1985) and hamsters (Craig and Rees, 1985). The deleterious effects of nicotine appear to be time dependent. Subcutaneous injection of nicotine resulted in decreased survival of rad dorsal flaps if given for 4 weeks preoperatively but not for 2 weeks (Forrest et al, 1987). Decreased distal and total capillary blood flow was seen as a result of exposure to nicotine for 5 weeks. The mechanism of tobacco or nicotine producing decreased flap survival is unknown, but may involve direct endothelial damage, vasoconstriction secondary to catecholamine release, or local concentrations of prostaglandins (Forrest et al, 1987).

Radiation delivered in a single dose of 1000 rads did not effect survival of dorsal rat flaps when given within 2 days before or after flap elevation (Nemiroff et al, 1985). Radiation given 12 to 104 weeks before surgery resulted in decreased flap viability in a ventrally based

porcine flank flap model (Young and Hopewell, 1983). A less important role for endarteritis obliterans in altered wound healing due to radiation has been proposed by two recent studies. In one, radiation of the recipient bed (5400 rads, completed 6 weeks preoperatively) was found to delay but not eliminate neovascularization of a rat epigastric flap (Clarke et al, 1985). In the second study, an intact delay phenomenon was demonstrated in irradiated rat dorsal flaps (Fisher et al, 1984).

The ischemic time occurring in the transfer of free flaps appears to hinder the flaps' ability to tolerate a second ischemic insult. Porcine ventral arterial island flaps initially exposed to 2 hours of ischemia followed by 12 hours of blood flow had a 50% survival rate after a subsequent 7.2 hours of "secondary ischemia" (Kerrigan et al, 1984). This is compared to 13 hours for the initial ischemic event needed to produce a 50% survival rate (Kerrigan and Daniel, 1982b).