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REVASCULARIZATION IN THE ORAL MUCOUS MEMBRANE FOLLOWING MICROVASCULAR INJURY*

by
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INTRODUCTION

Microvascular restoration following injury has always been taken for granted as an integrated process during wound healing. Consequently, few investigations have been conducted with the exception of a number of classical experiments on capillary proliferation in the rabbit's ear chamber. Additional experimental work to investigate fundamental principles of vascular rehabilitation would not only enlighten our concepts of wound healing of the oral mucous membrane but also promote our knowledge about development of collateral blood supply and revascularization of autogenous grafts.

This investigation was, therefore, designed to examine the process by which divided arteries and veins regain continuity and the concomitant development of collateral blood supply within the mucous membrane of the hamster cheek pouch.

A historical survey of regenerating blood vessels parallels that of wound healing and can be retraced for centuries. *Duhamel du Monceau* (1741, 1742) reported, according to *Meyer* (1852), the first experimental evidence

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that severed vessels do reconnect. *Van Swieton*, cited by *Marchand* (1901), confirmed these findings in 1757 and in addition observed that vessels reconnect end-to-end in such a way that arteries and veins seek their equivalence. Later investigations by *Hunter* (1835) failed to distinguish the mechanism by which the vessels reunited. He postulated, however, that the cut ends may either approach one another by some attractive forces or reconnect by means of a third vascular element formed within the intermediate coagulum.

Meyer (1852) emphasized that pre-existing vessels were the source of proliferating capillaries. *Clark* and *Clark* (1935, 1939) definitely established that newly forming vessels in the ear of the rabbit chamber, originated from the capillary sprouting of pre-existing vascular endothelium. In addition, these investigators observed that specific vessels differentiated, with age, into arterioles and venules to improve and control blood flow and facilitate capillary drainage. Other capillaries remained or regressed. *Moore* (1936) later corraborated these observations of capillary proliferation and differentiation in the ear chamber of dogs.

Expanding previous investigations, *Cliff* (1965) employed cinemicroscopy technique to follow wound healing in the rabbit's ear chamber. He observed and recorded by means of time-lapse-photography that vascular channels were re-established by means of mitotic division of existing functioning epithelial cells and »reutilization» of endothelial cells of regressing blood vessels. These findings supported earlier observations made by *Altschul* (1954, 1961), *Poole* (1958) and *Robertson et al.* (1959) that endothelial cells do multiply.

Cliff's (1965) sequential observations also clarified the controversy regarding the acquisition of a lumen by the proliferating vessels. Earlier electron microscopic investigations by Cliff (1963, 1965) and *Wiener* and *Spiro* (1962) had indicated that loculated spaces were present between the opposed endothelial cells of the capillary sprouts. It was therefore theorized that these spaces expanded and coalesced to form the vascular lumen. This hypothesis was confirmed by *Cliff's* (1965) *in vivo* experiments.

References to vascular regeneration and developing collateral blood supply in the oral mucous membrane following vessel interruption are limited, since previous work of this kind has dealt with major vessels of other structures. *Bellman et al.* (1959) performed one of the very few investigations on microvascular restorations following arteriolar occlusion. They utilized the anastomosing tri-arterial pattern of the rabbit ear and observed that proximal obstruction of the central artery led to compensatory functional expansion of the two lateral arteries. Obstruction of the central, as well as

one of the lateral arteries, led to substantial widening of the remaining trunk with simultaneous progressive enlargement of pre-existing collaterals. They suggested hemodynamic factors and inadequate tissue blood flow as primary factors for the development of collaterals. Later *Lambert et al.* (1961) observed the evolution of collateral channels following arterial ligation in different localizations of the rabbit ear. They demonstrated that the obstructed segment of the marginal artery was quickly bridged by enlarging arcades formed from the junction of smaller branches of the major artery. Direct end-to-end connections developed between the arteries within 50 days and concomitantly the temporary collaterals regressed.

In a subsequent *in vivo* investigation, *Lambert et al.* (1963) studied, by means of microangiographic and histologic methods, the reconnection of transected arteries and veins in the distal third of the rabbit ear. In four days they observed fine vascular communications across the line of incision and within a week some direct connections between the distal and proximal ends of the cut vessels. According to these investigators the vessels reunited by either collateral pathways, direct proliferation of severed vascular ends, or both.

The vasculature of the oral mucous membrane has yet to be investigated to elucidate its regeneration capacity following mechanical and thermal insults.

MATERIAL AND METHODS

The cheek pouch of forty-eight adult male Golden hamsters (*Mesocricetus auratus*) was selected as the experimental model. Under light general anesthesia (pentobarbital sodium 9 mg/100 gm body weight) the animal was placed in supine position on a specially constructed animal table (*Folke*, 1969). The cheek pouch was everted and then immobilized in a receptacle containing fluid (Fig. 1). The immersion fluid consisted of physiologic saline solution containing 1/2 % gelatin and was maintained at 37±1°C. Three areas of the cheek pouch displaying a smaller vein and accompanying artery were selected. Each site was tattooed with carbon for future identification of each vascular complex (Fig. 2a). Cine- and microphotographic recordings of vascular patterns and directions of blood flow were then conducted.

The vital microscopic equipment consisted of a Leitz Ortholux microscopic stand with a regular nose piece for conventional objectives and an Ultropak illuminator to accommodate dipping cone arrangements. The objectives employed were Leitz Ultropak 2.0X, 6.5X, 11X with ring condensor and 55X (water) without ring condensor. Dipping cones were routinely used with

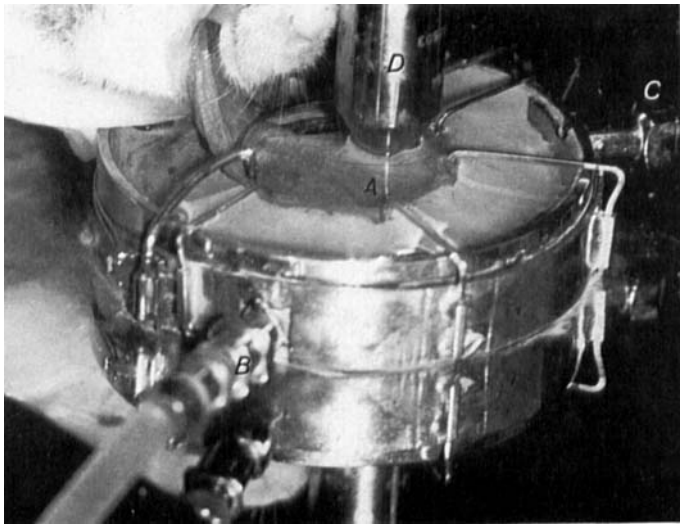


Fig. 1. The anesthetized hamster in place on the animal table with the cheek pouch stretched out and immobilized for observation (A). The physiologic saline or the test solution is continuously circulated through the receptacle via the inlet (B) and the outlet (C). Water immersion objective (D) in position for *in vivo* examination.

6.5X and 11X objectives. Permanent records of microscopic observation were obtained by means of a Paillard-Bolex H-16 M-X camera featuring a single frame device and running speeds from 12—64 fps permitting time-lapse-photography as well as slow-motion-cinephotography. To minimize vibrations, the camera was firmly mounted on the vertical camera carrier of a Leitz Aristophot stand and driven by an electric motor. Recordings were made on Kodak type 7242 Ektachrome EF film.

To obtain three different combinations of injured arteries and veins each vessel selected for observation was subsequently severed in one of the three following ways: bisectioning, longitudinal sectioning, or heat injury. The vascular lesions were performed by means of micromanipulators under a Zeiss Operation microscope at 40X magnification and transmitted light.

Complete bisection of the superficial vessels was accomplished with a modified Bard-Parker scalpel blade # 15, whereas longitudinal sectioning was made with a specially designed glass knife to obtain a reproducible cut of 3 mm. Thermal injury was induced by placing a heated thermister controlled silverloop (200 ± 1°C) in light contact with the epithelium above the vessels for approximately one second. The extent of the vascular injury was immediately recorded by cinephotomicroscopy.

Vascular healing was observed and recorded at 1, 3, 7, 14, 21 and 28 days.

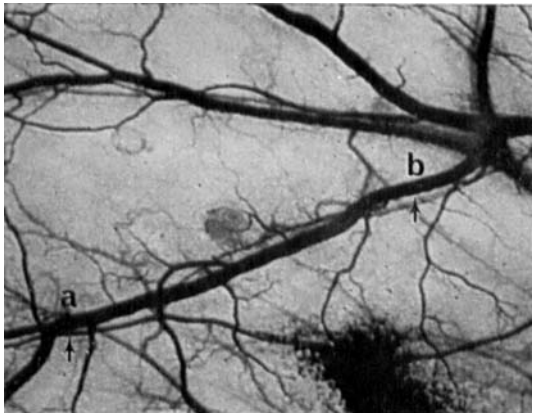


Fig. 2a. Small vein of the hamster cheek pouch prior to transverse sectioning in figures 2b and 2c. 12 ×.

Two animals received at each time point, an intravenous injection of carbon black* (0.1 ml/100 gm body weight) and Evans blue T-1824 (0.5 ml/100 gm body weight) to visualize intravascular damage or excessive permeability. Subsequent *in vivo* observations were continued for 2 hours.

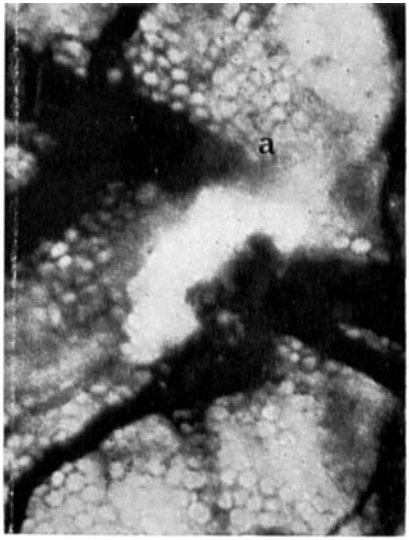
OBSERVATIONS

Vascular Transection

Vascular transection of the venous and adjacent arterial vessel produced profuse bleeding into the surrounding areolar tissue. Hemorrhage ceased within 10 to 15 minutes and developing intraluminal thrombi were evident in the venous ends opposite to the cut (Fig. 2a and b). The arteriolar vessels usually contracted immediately upon injury and, therefore, contributed little to the extravasation of blood. Simultaneously with the obliteration of the arteriolar ends, the blood flow reversed its direction in the distal vascular segment. This change in blood propagation was obviously related to the altered blood pressure differential following arteriolar disruption. The maintenance of circulation distal to the cut prevented ischemia of the tissue. This functioning circulation emphasis the clinical significance of the extensive collateral blood supply present in the hamster cheek pouch (*Folke*, 1969).

Venous vessels did not exhibit immediate change in diameter after surgical sectioning because blood flow and thrombi formation kept the lumen dis-

*Guenther-Wagner Pelikan-Werke. Hannover, Germany (Batch ## C 11/1431a).



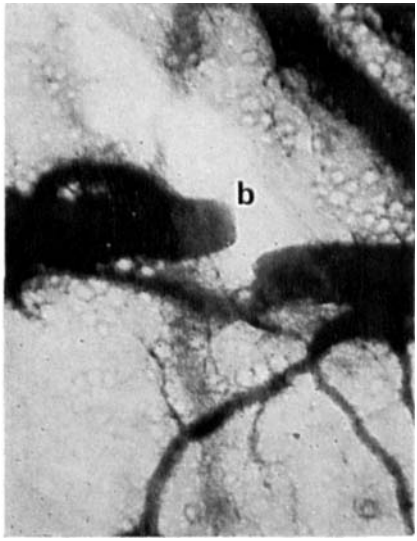


Fig. 2b. One hour following transection of vein at site a. 50×.
Fig. 2c. One hour following transection of vein at site b. Note the thrombus formation in each vascular end-piece. 50×.

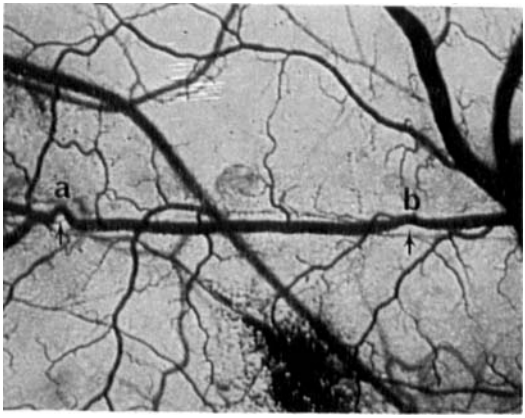


Fig. 2d. Seven days following transections of the small vein at site a and b in figure 2a. 12×.

tended. The arterial vessels, however, contracted several millimeters beyond the injured segment (Fig. 3b). Such vasoconstriction continued for 18 hours or more and was sometimes followed by distal, as well as proximal arterial degeneration. Such vascular disintegration stopped at the nearest unharmed

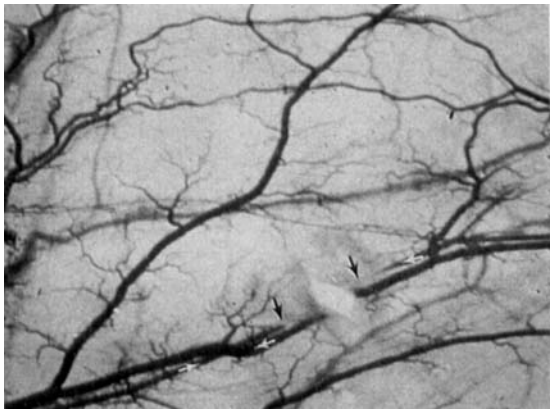


Fig. 3a. Small artery and vein prior to vascular transection. 12×.

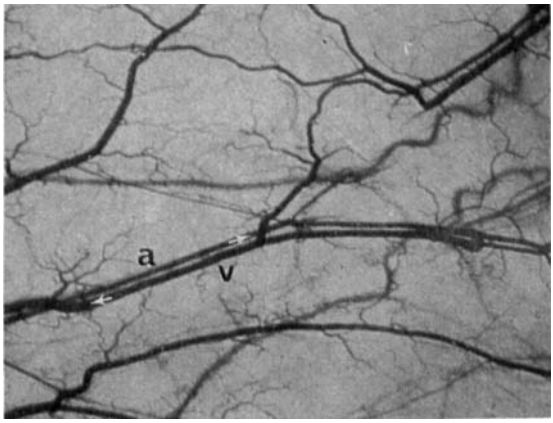


Fig. 3b. Vascular response immediately following transection of the vessels. Note the contraction of the arterial ends while the venous ends are occluded with thrombi. 12×.

arterial branch of the injured vessel. This produced a direct flow of blood and simultaneously eliminated vascular dead ends (Fig. 3c).

Two hours after transectioning, the gap was filled with blood, which quickly organized and fixed the blood vessels in position. In 24 hours the blood clot was well established. At this time there was no indication of vascular reconnections and severed vascular ends were still congested with thrombi and devoid of blood flow. Intravenously injected carbon black suspension escaped through the vascular ends proximal, as well as distal, to the line of incision demonstrating their incomplete obturation. Intramural

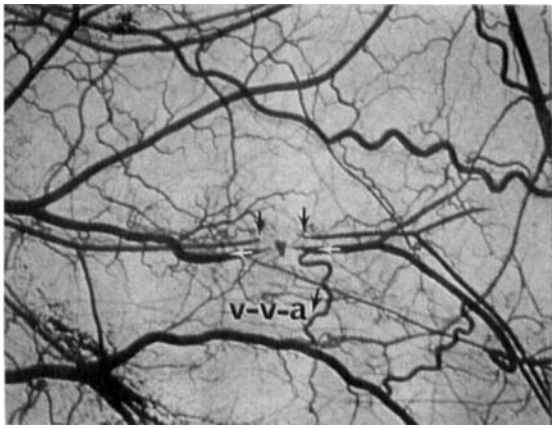


Fig. 3c. Seven days following vascular transection. Arterial and venous vessels remained disconnected. Arterial flow reversed its direction in the distal vascular segment while no change was observed in the venous counterparts. Note the enlarged collateral vessel (V-V anastomosis). 12×.

carbon deposits in vessels adjacent to the one injured indicate a concomitant alteration of their vascular integrity. Inflammation was present but remarkably modest considering the septic environment of the cheek pouch.

Rapid vascular healing was usually completed within seven days (Table I) by close adaptation of the sectioned ends or the formation of a short inter-

Table I.

The duration and frequency of vascular healing following different vascular injuries

Day of postoperative vascular healing	Transected Vessels		Longitudinally divided Vessels		Heat Injured Vessels	
	venous	arterial	venous	arterial*	venous	arterial
1	0	0	0	0	0	0
3	2	2	4	0	1	1
7	5	1	15	0	3	2
14	1	0	0	0	4	4
21	1	0	0	0	1	0
28	1	0	0	0	0	0
Number of vessels still disconnected	9	16	0	0	9	11
Total number of injured vessels	19	19	19	0	18	18

*Not attempted due to technical difficulties.

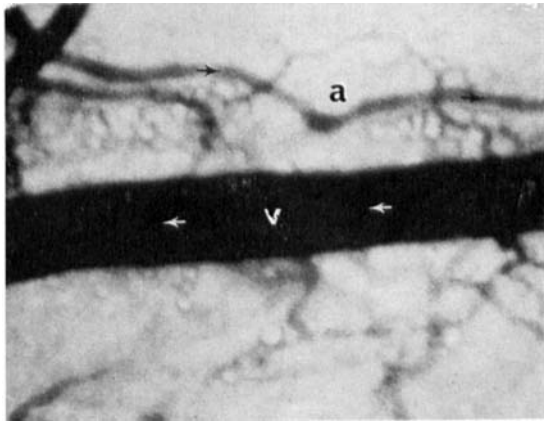


Fig. 4a. Arteriole and collecting vein prior to transection. 50×.

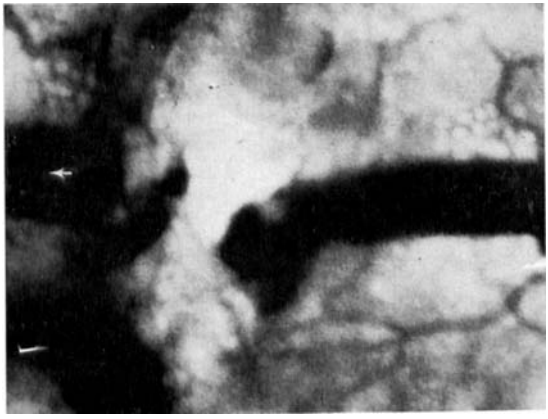


Fig. 4b. Immediately following transection. Blood flow continued uninterrupted in the proximal venous segment while stasis and thrombosis blocked the vascular end-piece distal to the line of incision. 50×.

mediate vascular piece within the blood clot. The lumen gradually became patent as the intraluminal thrombi blocking the flow of blood were dissolved. Concomitantly, collateral pathways had developed to accommodate the flow of blood (Fig. 3c). The healing vascular segment was usually constricted and had a somewhat irregular outline (Fig. 4c).

Only partly injured arterial vessels regained full continuity and blood flow by the third day (Table 1). Complete transection, however, prevented reconstitution of the original vessel (Figs. 3 and 4).

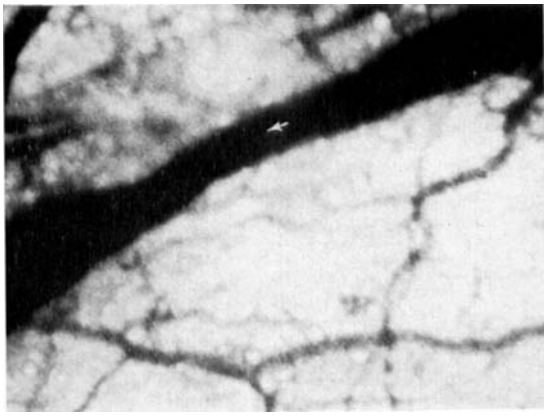


Fig. 4c. Seven days following injury. Venous connection was re-established by vascular proliferation within the blood clot. The vascular lumen remained obliterated. Note the smaller diameter of the bridging vascular segment. 50×.

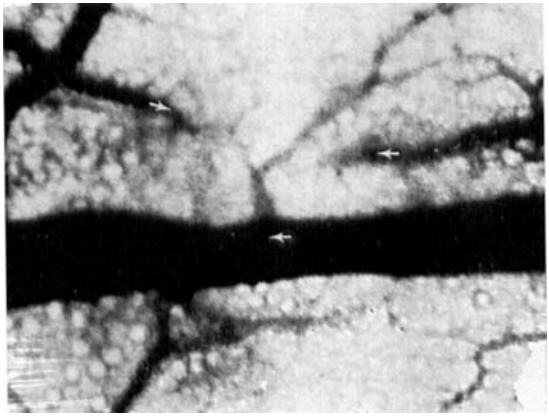


Fig. 4d. Twenty-eight days following transection. The arteriole remained disconnected. Blood flow was reversed in the distal segment due to pressure differentials. The venous lumen regained its patency and widened to almost the diameter of the original vein. 50×.

All healing vessels were extremely fragile during the first 4 to 7 days and vulnerable to hemorrhage or manipulation. Approximately half of the transected venous vessels reunited (Table I). Other vessels remained disconnected probably because the distance between the vascular ends was too wide to be bridged, or because sufficient collateral channels developed so quickly that a reconnection of the original vessel was unnecessary.

Direct shunts were not always present prior to venous injury, but developed immediately upon injury within the communicating vascular network adjacent

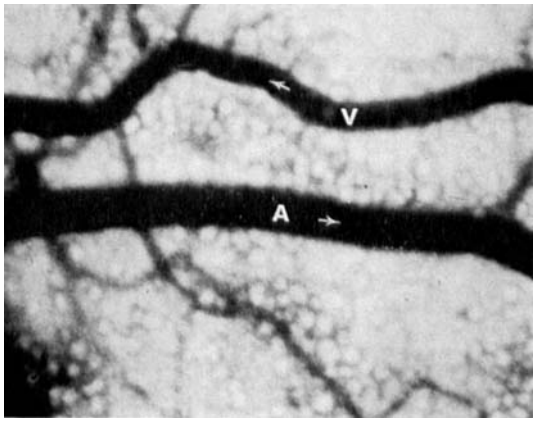


Fig. 5a. Arterial and venous vessels prior to transection. 50×.

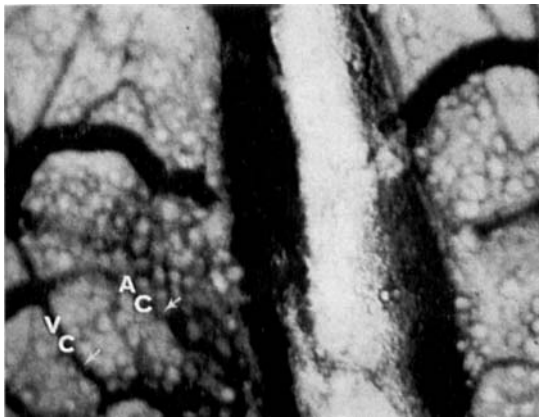


Fig. 5b. One hour after transection. Observe the typical contraction of the severed arterial ends while the venous ends maintained their diameter. Intravascular coagulation prevented further hemorrhage. Note the potential collaterals (VC) and (AC). 50×.

to the injury. These preferential channels continued to develop (Fig. 5) in response to functional demands and within two weeks attained approximately the same diameter as the parent vessel unless the latter regained its continuity and blood flow (Fig. 4). The development and regression of the collateral pathways were surprisingly rapid, and reflected closely the stagnation or vascular improvement respectively, within the sectioned vessel.

Vascular contours of healed vessels were never straight but usually curved in different patterns dependent upon the kind of vessel reconnection. Fairly straight channels developed in venous vessels having close end-to-end adap-

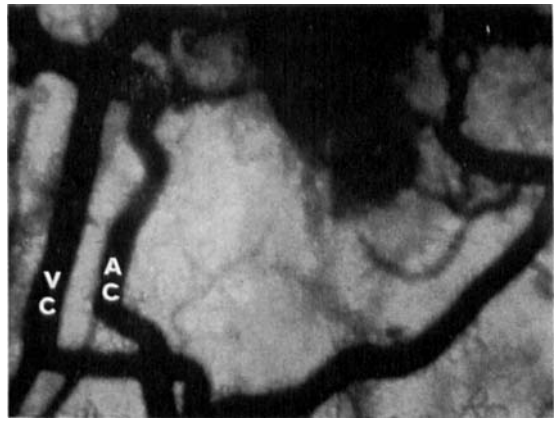


Fig. 5c. Three days following transection. Observe the rapid development of collateral vessels. Venous collateral (VC) shunting the blood across the injured site while arterial collateral (AC) directs the blood to adjacent arterial vessel. 50×.

Fig. 5d. Fourteen days after surgical sectioning. The venous vessel regained continuity by the enlargement of a pre-existing collateral vessel (V-V-A), while the arteriole was permanently disconnected. Note the widened A-A anastomosis (A-A-A) between the proximal segment and adjacent arteriole. 50×.

tations (Figs. 2c and 4) in contrast to a coiled vascular appearance resulting from a new vessel development within the blood clot (site a in Fig. 2d). Variable curved vascular connections were also seen in situations such as 1) where a pre-existing collateral branch was utilized to bridge the cut vessel (Fig. 5); and 2) where one of the two longitudinally bisected vascular segments re-established the continuity of the injured vascular vessel (Fig. 6).

The regeneration of vessels less than 30 microns in diameter did not follow the general principles described above. These vessels connected haphazardly into vascular plexi rather than restoring original vessels. It was not uncommon among the smaller vessels to have arterioles link up with venous vessels and thus bypass the capillary network. Restorations of this kind were never encountered in larger vessels because veins always connected to veins and arteries to arteries. Sometimes the healing of divided arterioles was characterized by pronounced vasodilatation and the appearance of solitary saccular formations. These features prevailed beyond the observation period of 28 days.

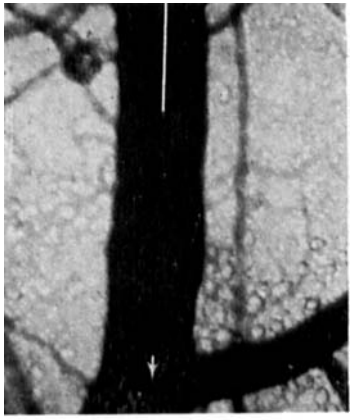
Longitudinal Sectioning

Longitudinally sectioned venous vessels healed in all of the experiments (Table I). Temporary thrombi were formed after vascular division, both within and between the two vascular segments (Fig. 6). These thrombi, however, did not seem to prevent seepage of blood from the distal to the proximal vascular segment. The flow increased steadily until vascular healing was completed within seven days. Usually the blood flow favored one of the longitudinally divided segments which seemed to contribute to its vascular restoration while the other segment degenerated. This path of vascular healing was characterized by a curved appearance (Fig. 6c). Its associated blood flow was rapidly restored, generally within 3 days. Vascular fragility and leakage of carbon particles were still evident at this time but regressed gradually with the completion of vascular healing about the seventh day (Fig. 6c). The newly formed vascular bridge was narrower than its parent vessel but demonstrated surprisingly little change in vessel diameter or shape during the subsequent three weeks.

Heat Injury

Heat injury of the cheek pouch produced an immediate blanching of the tissue (Fig. 7a). Venous and arterial vascular segments opposite to the injury developed a cone-like configuration and contained intraluminal thrombi (Fig. 7a). After a delay of 12 hours, bleeding appeared and a distinct blood clot was then formed in the heat injured area (Fig. 7b). The vessels were very fragile and carbon particles readily escaped into the perivascular space. In three days only one injured vessel had reconnected (Table I) and blood flow commenced across the injured area (Fig. 7b). In some experiments it seemed that newly formed vessels emerged directly from the tip of the

a.



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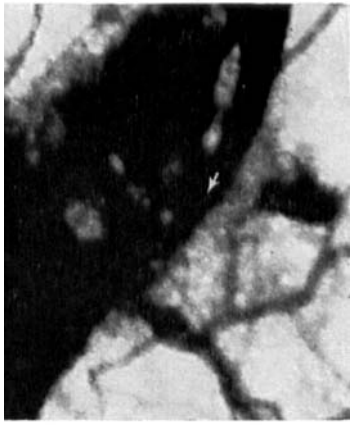

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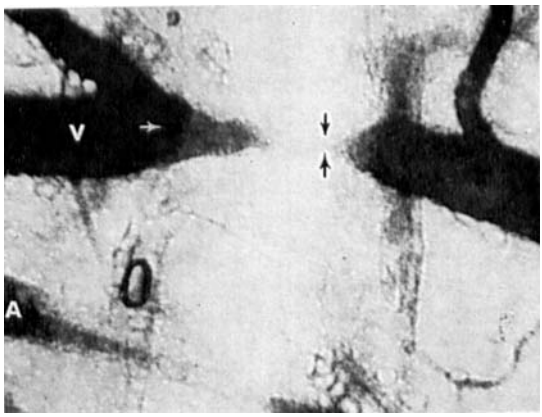

Fig. 7a. Accompanying artery and vein injured by mild heat. In addition to tissue blanching, the severed venous and arterial segments were found to react similarly with cone-shaped constrictions and intravascular coagulation. Observe the location of the proximal venous end (black arrows).

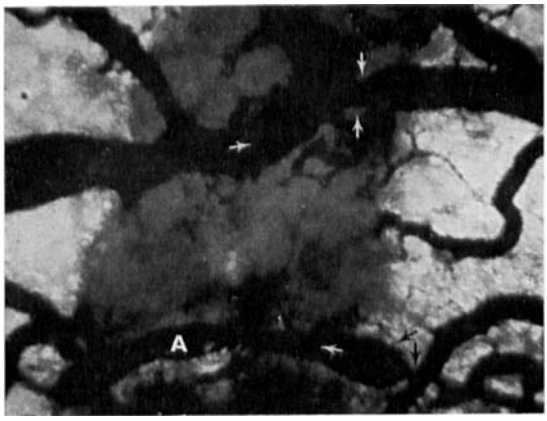

Fig. 7b. Three days after injury the vessels were still fragile and demonstrated hemorrhage and extravasation of carbon particles. The distal venous segment proliferated in the direction of blood flow and re-established vascular continuity and luminal patency. The proximal segment remained stationary. Note the sac-like dilatation of the arteriole in the area of injury and the peripheral vasoconstriction of the vessel (arrows).

Fig. 6a. Collecting vein of the hamster cheek pouch prior to longitudinal bisectioning along the indicated (solid) white line.

Fig. 6b. One hour following injury, the gap between the vascular segments was filled with coagulated blood. The bisected vascular segments bridged the uninjured proximal and distal vascular segments and facilitated vascular regeneration.

Fig. 6c. Seven days following injury. The right bisected segment was utilized as the core of vascular regeneration. Note the curved configuration of the reconstituted vessel and the degeneration and disappearance of the left segment.

injured segment, while in other instances a wide, direct bridging pathway formed immediately at the point of venous interruption. The regenerating arteriolar segment was greatly distended in a sac-like configuration within the area of thermal injury. Beyond the damaged site, however, the arteriolar vessel displayed marked constriction (Fig. 7g). Carbon deposition within the injured vessel walls and extravasation into surrounding tissue were still prominent but decreased gradually with maturation of the vessels.

In 6 to 14 days the blood clot disappeared and most vessels regained their continuity (Table I). Once established the vascular pattern remained the same throughout the experimental interval. In addition to some modifications of the vascular anatomy, the most conspicuous evidence of a previous injury was the obvious reduction in diameter of the venous vessels to approximately 50 to 75 % of their original width. No obvious scar formation occurred in the cheek pouch and the burned site could only by detected by comparing the vasculature before and after heat injury.

DISCUSSION

The most striking observation of the present investigation was the ability and speed by which the majority of injured vessels reconnected and healed. It was anticipated that factors such as oral bacteria, food debris and continuous movement of the hamster cheek pouch would prolong wound healing and interfere particularly with vascular restoration. However, vessels reconstituted remarkably fast in this mucous membrane especially when compared to observations of similar studies in the rabbit ear (*Lambert et al.*, 1963).

The prognosis of complete vascular healing differed with the insult and the type of vessel involved. Transecting venous vessels within the cheek pouch did not always result in reconnections (Table I). It was, therefore, difficult to predict the outcome of this kind of vascular injury. Although various factors such as blood clot formation, granulation tissue, collateral channels, hemodynamic forces, and the distance of vessel separation influenced vascular regeneration, it was not the prime concern of the present investigation to assess their individual effects. Nevertheless, the establishment and differentiation of the blood clot into granulation tissue was found to be a prerequisite to produce adaptation of the vascular ends. The continuous observations of the heat-injured venous vessels supported this hypothesis as no vascular proliferation was evident until the blood clot was demonstrable (Fig. 7).

At the time the blood clot was established, the thrombotic venous ends displayed rapid fibrinolytic activity. Simultaneous vascular proliferation of

the end pieces occurred, producing a broad pathway bridging the interrupted vessel. Whether or not the inception of this constructive process was associated with inflammatory reactions (*Clark & Clark*, 1939), hemodynamic forces (*Thoma*, 1873; *Clark*, 1918), or was just related to the perivascular blood clot (*Chalkley et al.*, 1946), can only be speculated upon at this time. Previous work by *Bellman et al.* (1959) had indicated that the reunion of arteries was accomplished by gradual enlargement of either pre-existing or newly formed minute vessels. According to the same investigators the latter vessels are induced by the inflammatory response accompanying wound healing. *Lambert et al.* (1963) supported the theory of microvessels forming the interconnecting link and presented evidence that several channels bridged the arterial ends across the line of incision. Present observations are only in partial agreement with their findings because the comparatively smaller venous vessels of the hamster cheek pouch reunited with the development of only one channel. This intermediate vascular segment was established within the blood clot and not derived from pre-existing vessels unless a typical collateral pattern circumvented the line of incision. Based on observations of arterial vascular healing in the rabbit ear (*Bellman et al.*, 1959 and *Lambert et al.*, 1963) and venous restoration in the hamster cheek pouch, it is questionable whether the discrepancies reported are related to different microvascular anatomy of each individual structure or whether it is a matter of vessel specificity or size. Present knowledge indicates that these factors are definitely integrated.

The impact of hemodynamic forces seemed beneficial in creating vascular patency by dissolving and/or detaching intraluminal conglomerations. This observation tends to support previous assumptions made by *Thoma* (1873), *Clark* (1918) and *Lambert et al.* (1963). Whether endothelial cells proliferated into the blood clot as a solid cord (*Billroth*, 1856; *Cliff*, 1963) prior to the establishment of the vascular lumen cannot be determined without ultrastructural documentation. However, the rapid formation of a relatively large bridging channel between the severed vascular ends, suggest a possibility of an initially temporary vessel wall formed by a condensation of connective tissue cells. The endothelial lining of the lumen could then possibly be a secondary event either through ingrowth of endothelial cells or their differentiation from mesenchymal precursor cells.

Increasing the distance between the cut vascular ends minimizes the likelihood of vascular reunion and promotes the establishment of a collateral pathway. In either case, it is obvious that the blood flow has a direct effect upon the vessel *per se* by widening the lumen and stimulating the vessel wall to differentiate and become morphologically similar to the vessel it joins.

This pattern of vascular healing was seldom paralleled by the transected arterioles. Their low incidence of reconnection (Table I) could be ascribed to several factors. The immediate constriction of the arterioles upon injury was an effective means of reducing excessive blood loss, but this diminished the prospects for a vascular reconnection. Proximal and distal retraction of the arterial ends widened the gap more so than that of the accompanying transected venous vessel which precluded arteriolar reconnection in all complete transection experiments (Figs. 3 and 4). All arterial vessels distal to the line of transection quickly reversed their blood flow, providing an additional source of blood to the injured area. This mechanism might be relevant to the process of wound healing. In any case, it substantiated the existence and importance of the arterial connecting network in the hamster cheek pouch (*Folke*, 1969) as well as the effect of hemodynamic pressure differentials.

The three cases of arteriolar reunion observed (Table I) were most likely due to incomplete transection. This explanation was, at first, difficult to substantiate but it became evident from subsequent studies of heat injured and longitudinally sectioned vessels that any remaining continuity enhanced and secured vascular re-establishment.

The conclusion that vessel contingency following vascular injury promotes vascular restoration, is substantiated by the results of longitudinal sectioning. Each vein insulted in this manner healed rapidly. This healing was usually faster than that following other vascular injuries whether the vessel healed *restitutio ad integrum* or whether it restored one of the bisected vascular segments (Fig. 6). It was evident that the longitudinal cut was less traumatic to the vessel in view of post-surgical vascular regeneration.

Following the selected heat injury, arteriolar vessels usually retained some viable connections. Although constricted at first, these vessels regained their lumen in a sausage-like vasodilated fashion. This configuration developed when the expanded ends abruptly joined constricted arterioles (Fig. 7b). This characteristic vascular abnormality remained throughout the experimental interval of four weeks and distinctly indicated the site of prior vascular injury. The cause of this vascular dilatation remains unexplained, but it is realistic to assume that the applied heat may have damaged the contractile tissue within the vessel wall or disturbed the innervation of the vascular smooth muscles.

Heat insult also manifested instant constriction of venous vessels and terminal coagulation of vascular ends facing the affected area (Fig. 7a). During healing it was intriguing to see that the distal vascular end proliferated in the direction of blood flow whereas the proximal venous end remained

stationary. This observation supports the hypothesis that intraluminal blood pressure influences vascular proliferation.

Restoration of the heat-injured venous vessels closely paralleled the results of transection (Table I) and was equally unpredictable. Although the insult was followed by a prolonged stasis and ischemia, the vascular ends did not lose their capacity to proliferate at the time blood clot formation was finally established. Actually, vessel regeneration seemed to be accelerated when considering the amount of revascularization accomplished within seven days.

From these various experiments one cannot form any conclusion as to the nature of the stimulus evoking this vascular growth, but previous investigations on retinal revascularization have suggested that endothelial cell proliferation is directly related to the presence of oxygen. This has been proposed by *Ashton* (1961) who considers the presence of living tissue, low oxygen tension and poor venous drainage to be the governing factors for vessel growth. Since vascular repair and connective tissue proliferation have been associated with increasing levels of histamine as well (*Boyd & Smith*, 1959), it is conceivable that the same concept is applicable to the cheek pouch. Although the role of histamine in wound healing is still unclear, the problem seems relevant enough to pursue in additional studies.

The healing of the cheek pouch paralleled revascularization of the major vessels and was completed within 8 to 10 days. This observation coincided with previous reports by *Shulman et al.* (1954). Their observation that the burned site was undetectable could not be supported in this study since the vasoconstriction and modified vascular pattern characterized the area of previous injury.

Fragility and immaturity of healing vessels were demonstrated with carbon black suspensions. Evans blue, on the other hand, permeated too diffusely to resolve discrete breaks in the vascular wall. Extravasation of erythrocytes and carbon particles was inevitable during the first few days of healing, particularly if light pressure was exerted upon the vessel. This observation is not remarkable since recently formed or forming vessels are known to be fragile (*Sandison*, 1928; *Clark & Clark*, 1939; *Ebert et al.*, 1939; *Cliff*, 1963) It is interesting, however, that heatinjured vessels, in contrast to those mechanically damaged, are fragile and susceptible to hemorrhage and extravasation of carbon particles over a longer period of time.

With vascular restoration carbon particles were gradually trapped within the vessel wall, indicating a leakage of plasma rather than whole blood. This subsided within 7 to 10 days, indicative of the rapid reconstitution of vessel wall integrity. *Schoefl* (1963) reported similar data based on ultrastructural examinations of proliferating capillaries in the cremaster muscle, that

the constant motion of the endothelial cells was mainly responsible for the extravascular escape of carbon particles.

The above hypothesis may also explain the vascular carbon labeling of the developing collateral venous channels. This carbon deposition was of the intramural variety which *Cotran* (1967) considers to be the result of mild heat or vascular mediators such as histamine and serotonin. Although it is quite conceivable that many vessels were influenced by permeability increasing factors during the acute stages of vascular healing, it is difficult to apply this explanation to developing collateral channels. The reason is that they demonstrated vascular labeling over a longer period of time than the adjacent venous vessels. The author consequently believes that the increasing flow in the collateral channels induces, concomitantly with the luminal widening, a migration and proliferation of endothelial cells which results in numerous true interendothelial gaps.

Collateral pathways are frequently established in vascular injuries to provide a continuous flow of blood in certain organs and tissues. The vasculature of the cheek pouch is well suited for such functional redistributions of blood flow since it has an abundance of communicating venous and arterial vessels (*Folke*, 1969). Considering that collateral channels are proposed to develop from either pre-existing or from newly formed vessels, the present investigation supports the former concept. The enlargement of the lumen of the pre-existing vessels seemed to be directly related to hemodynamic forces which has also been substantiated in the rabbit ear (*Lambert et al.*, 1963).

Since blood was rechanneled in a variety of ways upon venous transection, it is unclear which factors determine the selection of certain bridging collateral channels. Unless a direct loop was available to accommodate blood flow around the line of incision, blood usually followed a more elaborate course via interconnecting venous side branches before entering the proximal vascular segment. Sometimes blood was shunted directly into another collecting vein (Fig. 3). The anatomical structure facilitates such a redistribution of blood, but the preferred pathway in each given state was presumably the one offering the least resistance to flow. As more functionally convenient collaterals developed it appeared that the flow of blood gradually favored these channels. This was dramatically exemplified when the original vessel regained its continuity and the collateral connection began to regress. The same behavior and temporary function has also been described for collateral vessels in the rabbit ear (*Lambert et al.*, 1961).

Transected arterioles in contradistinction to venous vessels, neither reunited end-to-end nor developed a substantial collateral blood supply. Nevertheless the blood flow continued apparently undisturbed and it is possible

that blood is propagated so easily into the capillary network that there is no need for a re-establishment of the smaller arterioles. Although larger distributing arteries do reconnect (*Bellman, et al.*, 1959; *Lambert et al.*, 1961; *Lambert et al.*, 1963), the reunion is accomplished through collateral vessels rather than a newly formed intermediate segment. This discrepancy has yet to be elucidated.

Although arteries and veins in the cheek pouch are closely associated and parallel, arterio-venous connections were never established during vascular healing of the transected vessels. *Lambert et al.* (1963) made similar observations in the rabbit ear. This confirmed previous clinical experiences that arteriolar vessels do not join venous vessels or vice versa during wound healing. However, this concept did not always apply to the microvessels since sectioning of smaller arteriolar and venular vessels ($<20\mu$) sometimes led to direct arterio-venous connections.

Clinical implications of the present investigation are particularly significant for surgical interventions of the oral mucous membrane. Thus, it is reasonable to suggest that the vessels should sustain as little trauma as possible during surgical procedures. Applying this principle would mean that all surgical incisions should be made parallel and not perpendicular to the long axis of the major vessels whenever possible. This would decrease the number of accidentally transected vessels and promote the prognosis for rapid vascular re-establishment. It is, therefore, suggested that microvascular anatomy become one of the criteria in the design of surgical incisions and flaps.

Prolonged and unpredictable healing of the vasculature following heat injury contraindicates the routine use of electrosurgical techniques in the oral cavity. Unless there are special indications, the latter application should be avoided in favor of sharp surgical incisions.

SUMMARY

Venous and arterial vascular healing was investigated in both cheek pouches of forty-eight Golden hamsters following transection, longitudinal sectioning and heat injury. Cinemicrophotographic recordings were performed at 1, 3, 7, 14, 21 and 28 days to document vascular reconnection, reconstitution and subsequent direction of blood flow. Intravenous injections of a carbon black suspension and Evans blue were undertaken at the same time points to visualize, *in vivo*, the fragility and permeability of the healing vessels. The following observations were made:

1) Following any of the three induced vascular insults thrombosis plays a major part in obliterating the terminal segments of the veins while vascular

contraction was responsible for constricting the severed ends of the arterioles.

2) The venous vessel proximal or distal to the vascular interference did not demonstrate any change in diameter while arteriolar constriction was evident for several millimeters from the injured end. Vasoconstriction prevailed up to 18 hours.

3) Two hours after transverse or longitudinal sectioning the gap was filled with coagulated blood, which fixed the vessels in position. Vessel fragility and permeability were pronounced during the first three days of healing. Mechanical insults such as gentle pressure or stretching resulted, therefore, in renewed hemorrhage.

4) Most of the injured venous ends that reconnected regained their patency and flow in 3 to 7 days. The longitudinally sectioned venous vessels healed generally faster than the other two induced vascular injuries and the damaged vascular trunk was restored in all instances. However, the prognosis of vascular regeneration was unpredictable following transection and heat injury.

5) In transections, where the diastema between the vascular ends became too wide, collateral pathways were permanently utilized. Unless a direct anastomosing loop was already in existence prior to venous injury, small communicating vessels quickly enlarged and created a preferential channel to bridge the severed vascular section. The enlargement of collaterals was rapid and presumably controlled by physical forces. Depending upon the reconstitution of the injured vessel the collaterals would either remain the same or gradually decrease in size.

6) In none of the 36 experiments conducted did a transected arterial vessel connect to an adjacent venous vessel.

7) Immediately following heat injury the tissue became ischemic. Thrombosis and some vasoconstriction of the venous vessels were noticed while the arteriolar contraction was pronounced. Twelve hours following the vascular insult, the injured area was highly vascular and bled easily. Vascular continuity and blood flow were restored in 5 to 14 days.

RÉSUMÉ

REVASCULARISATION DANS LA MUQUEUSE BUCCALE APRÈS LÉSION MICROVASCULAIRE

La réparation vasculaire veineuse et artérielle a été étudiée dans les deux abajoues de 48 hamsters dorés après coupure transversale, coupure longitudinale et lésion par brûlure. Des enregistrements cinémicrophotographiques ont été faits au bout de 1, 3, 7, 14, 21 et 28 jours pour montrer le rétablisse-

ment des connexions vasculaires, et la reconstitution et la direction prise ensuite par la circulation du sang. Des injections intra-veineuses de noir de carbone en suspension et de bleu d'Evans ont été pratiquées à ces mêmes moments pour mettre en lumière *in vivo* la fragilité et la perméabilité des vaisseaux en voie de guérison. Les observations suivantes ont été faites:

1) Quel que fût le type de la lésion vasculaire produite, la thrombose jouait un rôle capital en oblitérant les segments terminaux des veines, tandis qu'une contraction vasculaire était responsable de la constriction des extrémités lésées des artérioles.

2) Aucun changement du diamètre vasculaire veineux, ni du côté proximal, ni du côté distal de la lésion vasculaire, n'a été observé, tandis que la constriction des artérioles apparaissait nettement jusqu' à plusieurs millimètres de l'extrémité lésée. La vaso-constriction persistait jusqu'à 18 heures.

3) Deux heures après la coupure transversale ou longitudinale, l'interstice était rempli de sang coagulé, qui maintenait les vaisseaux en position. Il existait une fragilité et une perméabilité vasculaire prononcées pendant les trois premiers jours de la période de guérison. C'est pourquoi des insultes mécaniques telles qu'une légère pression ou distension provoquaient une reprise de l'hémorragie.

4) Dans la plupart des extrémités veineuses lésées dont la connexion se refaisait, le passage et la circulation étaient rétablis en 3 à 7 jours. La guérison veineuse après coupure longitudinale se faisait en général plus rapidement que celle des deux autres types de lésions vasculaires, et, dans tous les cas, la reconstitution du tronc vasculaire lésé avait lieu. Cependant, il était impossible de prévoir le pronostic de la régénération vasculaire après coupure transversale et lésion par la chaleur.

5) Dans les coupures transversales, lorsque les interstices entre les extrémités vasculaires devenaient trop larges, des voies collatérales étaient définitivement utilisées. A moins qu'il n'y ait déjà une boucle d'anastomose directe avant la lésion veineuse, de petits vaisseaux communiquant s'agrandissaient rapidement et formaient un canal préférentiel reliant les extrémités vasculaires lésées par coupure. L'agrandissement des branches collatérales se faisait rapidement et était probablement sous la dépendance de forces physiques. Suivant la reconstitution du vaisseau lésé, les collatérales persisteront inchangées ou diminueront de grandeur.

6) Dans aucune des 36 expériences faites, un vaisseau artériel ayant subi une coupure transversale ne s'est relié à un vaisseau veineux adjacent.

7) Immédiatement après les lésions par brûlures, les tissus devenaient ischémiques. Dans les vaisseaux veineux, on notaient des thromboses et une certaine vaso-constriction, tandis que la contraction des artérioles

était marquée. Douze heures après la lésion vasculaire, la zone lésée était fortement vascularisée et saignait facilement. La continuité vasculaire et la circulation du sang étaient rétablies en 5 à 14 jours.

ZUSAMMENFASSUNG

REVASKULARISIERUNG DER MUNDSCHLEIMHAUT NACH MIKROVASKULÄREN VERLETZUNGEN

Die Heilung von kleinen Venen und Arterien wurde an beiden Hamstertaschen von 48 Goldhamstern untersucht, nachdem Transsektionen, Längssektionen und Hitzeverletzungen durchgeführt worden waren. Cinemicrophotographische Aufzeichnungen wurden nach 1, 3, 7, 14, 21 und 28 Tagen vorgenommen, um die vaskuläre Wiedervereinigung sowie die Wiederherstellung und Richtung des Blutstromes zu dokumentieren. Zu denselben Zeitpunkten wurden intravenöse Injektionen mit einer kohlschwarzen Suspension (Satz C1431, Pelikan Werke, Hannover) sowie mit Evans blue vorgenommen um *in vivo* die Fragilität und Permeabilität der Gefässe im Stadium der Heilung sichtbar zu machen. Hierbei wurden folgende Beobachtungen gemacht:

1. Anschliessend an jede der drei verursachten vaskulären Verletzungen spielt die Trombose die Hauptrolle bei dem Verschluss der Endstücke der Venen, während die vaskuläre Kontraktion für den Verschluss der verletzten Enden der Arterien verantwortlich war.

2. Das venöse Gefäss, proximal oder distal von der vaskulären Verletzung zeigte keinen Wechsel im Durchmesser des Gefässes, während ein Zusammenziehen der Arteriolen, mehrere mm von dem verletzten Ende entfernt, festgestellt wurde. Die Vasokonstriktion hielt 18 Stunden lang an.

3. Zwei Stunden nach den transversalen oder longitudinalen Schnitten war der Zwischenraum mit einem Blutkoagulum gefüllt, welches die Gefässe in ihrer Lage fixierte. Die Fragilität und Permeabilität der Gefässe war verstärkt während den ersten drei Tagen der Heilung. Mechanische Traumen wie leichter Druck oder Strecken resultierte daher in einer erneuten Blutung.

4. Die meisten der verletzten Enden der Venen die sich wiedervereinigten gewannen ihre Durchgängigkeit und ihren Durchfluss in 3—7 Tagen wieder. Die längsdurchtrennten venösen Gefässe heilten im Allgemeinen schneller als bei den anderen zwei verursachten vaskulären Verletzungen und der beschädigte Gefässtamm wurde in allen Fällen wiederhergestellt. Die Prognosis für die vaskuläre Regeneration nach der Transsektions- und Hitzeverletzung dagegen, war nicht vorauszusehen.

5. In Transsektionen, in welchen der Zwischenraum zwischen den Gefässenden zu weit wurde, wurden kollaterale Seitenwege permanent benutzt. Wenn nicht eine direkte Anastomoseschlinge bereits vor der Verletzung der Vene existierte, so vergrösserte sich die Verbindungsgefässe sehr rasch und bildeten einen bevorzugten Kanal, um den verletzten Gefässanteil zu überbrücken. Die Vergrösserung der kollateralen Gefässe war schnell und vermutlich durch physische Einwirkungen kontrolliert. Ja nach der Wiederherstellung der verletzten Gefässe blieben die kollateralen Gefässe in derselben Grösse bestehen oder sie wurden nach und nach kleiner.

6. In keinem der durchgeführten 36 Experimente vereinigte sich ein durchtrenntes arterielles Gefäss mit einem benachbarten venösen Gefäss.

7. Sofort nach der Verletzung mit Hitze wurde das Gewebe anämisch. Es wurden Trombose und eine gewisse Vasokonstriktion der venösen Gefässe beobachtet, während die Kontraktion der Arteriolen ausgeprägt war. Zwölf Stunden nach der vaskulären Verletzung war der betroffene Gewebeabschnitt hochgradig vaskularisiert und neigte leicht zur Blutung. Gefässvereinigung und Blutstrom waren in 5—14 Tagen wieder hergestellt.

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