The American Journal of PATHOLOGY

MARCH 1969 • Volume 54, Number 3

Ischemia of the Skin

Electron Microscopic Study of Vascular Injury

Kaethe Willms-Kretschmer, M.D., and Guido Majno, M.D.*

RECENT EXPERIMENTAL EVIDENCE has shown that two major factors determine the final outcome of a temporary ischemic insult: the resistance of parenchymal cells to ischemia and the ability of the blood vessels to resume and maintain blood flow when the cause of ischemia is removed.[1-4] The latter factor, though obviously important, has been little explored. From the data available, it is clear that during the ischemic period, and shortly thereafter, a number of changes may take place in the lumen of the blood vessels, and in their walls or around them, with the overall result of restricting the flow of blood. In the brain, an important mechanism in the pathogenesis of ischemic injury is the swelling of perivascular glial cells, which compress the lumen of the small blood vessels.[3] In other tissues there are no cells comparable in position to the perivascular glia, but many other changes could take place with a similar final result. The purpose of our experiments was to study the overall effect of ischemia on the skin and then to focus especially on the effects of ischemia on the blood vessels.

This approach should be especially pertinent to the pathophysiology of skin grafts and skin flaps, the survival of which depends in large measure on reflow in vessels that have suffered a period of ischemia.[5-7]

From the Department of Pathology, Harvard Medical School, Boston, Mass.

Supported by Grants AM-10829 and HE-08794 from the U. S. Public Health Service.
Accepted for publication Nov. 29, 1968.

Address for reprint requests: Dr. Willms-Kretschmer, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Apartado Postal No. 70228, Ciudad Universitaria 20, D. F., México.

* Present address: Institut de Pathologie, 40 Boulevard de la Cluse, Genève, Switzerland.

At the time our experiments were undertaken, we were unable to locate a single reference to the study of ischemia in the skin; a partial exception is represented by studies on the pathogenesis of decubital ulcers, which focused primarily on the necrosis of muscle.[8,9] In the meantime, Selye published a brief study of rat skin ischemia with the main purpose of investigating the effect of stress and other factors on the survival of the skin after this insult.[10]

Material and Methods

Two models were used: (1) the dorsal skin of the rat, which consists of epidermis, dermis, panniculus adiposus (adipose tissue), panniculus carnosus (intrinsic striated muscle), and a thin layer of subcutaneous tissue; and (2) the rabbit ear, on which the skin is very thin and consists only of epidermis and dermis—a large number of vessels run in the deep part of the dermis, with only a thin layer of connective tissue separating them from the cartilage.

Experiments with Rats

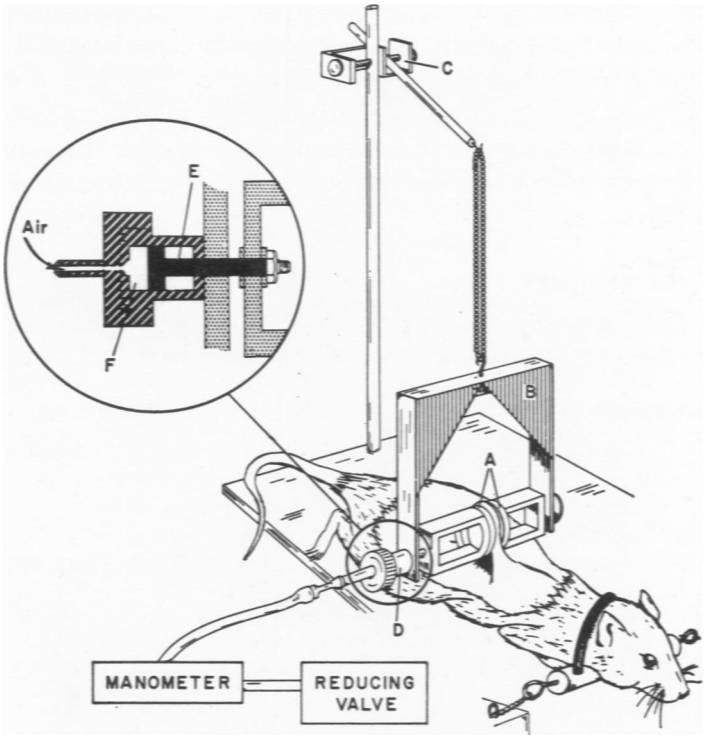
A total of 67 animals of the Sprague-Dawley strain, weighing 150–300 gm., were used. The back of the rat was shaved and depilated with Nair (Carter-Wallace, Inc.), and a tranquilizing dose of Thorazine (chlorpromazine hydrochloride, 1 mg./100 gm., kindly supplied by Smith, Kline and French) was injected intraperitoneally 20 min. before the experiment. The rat was then fitted with a loose neck harness and placed on a restraining board as shown in Text-fig. 1. A fold of skin from the back was slipped into a clamp consisting of two parallel rings—one fixed, the other attached to a compression chamber whereby the skin fold could be submitted to pressures up to 4.2 kg./sq. cm. (Harvard Apparatus Co.). The pressure was supplied by a nitrogen tank and controlled by manometer (Text-fig. 1). The clamp was tightened by rapidly raising the pressure to 1.9–2.1 kg./sq. cm., at which point the skin within the rings was cut off completely from the peripheral circulation. After 1–8 hr. the clamp was released, and after an additional interval of 0–48 hr. the rats were sacrificed by clamping the thoracic vessels.

Injection of Vascular Markers

The markers used were Evans blue (30 mg./kg., 2.5% in 0.45% saline) and colloidal carbon black in a dose of 0.1 cc./100 gm. (Pelikan Werke, Hannover, West Germany, Batch C11/1431a). One or the other of these preparations was injected I.V. (1) just before applying the skin clamp, (2) just prior to releasing the skin clamp, or (3) 1–2 hr. after release.

Determination of Water Content in Skin

Under ether anesthesia, square pieces of skin approximately 5 mm. on edge were taken from the center of each lesion, immediately weighed, dried at 110°C. for at least 72 hr., and reweighed; the water content was expressed as a percentage of the initial fresh weight. Samples from both ischemic lesions were taken after 2 and 6 hr. of ischemia (6 animals) and after 8 hr. of ischemia (4 animals). All these samples were taken 1½ hr. after release of the skin clamp. Two pieces of normal skin from each experimental animal were used as controls.



TEXT-FIG. 1. Drawing of clamp applied to rat skin. Two aluminum rings (*A*) are attached to a frame (*B*) suspended from a metal rod (*C*) of adjustable height. A fold of dorsal skin is slipped between the rings; Left ring is connected to pressure drum (*D*) and driven by piston (*E*, inset) when compressed air is let into chamber (*F*). Pressure is regulated by manometer (range 15–60 psi). Blood circulation within skin fold was occluded completely at 28–30 psi (approx. 2 kg./sq. cm. = 1.8 atm. total pressure). Actual dimensions: rings, O.D. 7/16 in., I.D. 5/8 in.; frame 2¼ × 2¼ × ½ in.

Experiments on Rabbit Ears

The tip of the rabbit ear is thin enough that after depilation the vascular tree can be seen by transillumination and photographed over a viewing box; therefore, this model was used especially to study the direct effect of the pressure ring on the underlying vessels. A total of 15 albino rabbits weighing 1.6–3.0 kg. were used. Each animal was placed in a wooden box with his head exposed; no tranquilizer or anesthetic was given. The tip of the right ear was shaved, depilated, slipped between the rings of the clamp, and adjusted so that the margin of the ear would overlap the margin of the clamp by at least 5 mm. A pressure of 2.1 kg./sq. cm. was then applied. At the end of each ischemic period the animals were anesthetized with I.V. Diabutal (sodium pentobarbital, 100 mg./kg., Diamond Laboratories, Inc.). The marginal vein of the other ear was cannulated to facilitate administration of markers or of further anesthetic. At the end of the ischemic period, colloidal carbon was injected (0.1 cc./100 gm.); immediately thereafter the clamp was released, and the corresponding area was adjusted rapidly over a specially built viewing box previously centered under a camera lens. Transillumination was provided by a "cold-light" Zircon-arc lamp (Fish-Schurman Corp.) aimed at a mirror

which could be adjusted for maximum lighting of the viewing surface (a pane of ¼-in. "milky" glass). Beginning 1 min. or less after flow was re-established, serial photographs were taken at 2- and 5-min. intervals to document the time required for circulation in various vessels to resume. The animal was sacrificed as soon as flow had been re-established completely and the area cleared of carbon; appropriate samples of tissue were taken immediately for light and electron microscopic studies. Two animals subjected to 8 hr. of ischemia were kept alive 5 days for the study of long-term effects.

Light Microscopy

Pieces of skin from all experiments were fixed in 10% buffered formalin, Smith Atkinson's mast cell fixative,[11] or Bouin's fixative. Paraffin sections were stained with hematoxylin and eosin, PTAH, and Smith-Atkinson mast cell stain.[11]

Electron Microscopy

Pieces of skin were obtained from animals under general anesthesia or immediately after sacrifice. A strip of skin was cut from the lesion with a razor and placed in a pool of fixative for 2–3 min. Whole thickness blocks of skin, 1 mm. square, were then cut from the center of the lesion and fixed for 2–3 hr. in 2.5% glutaraldehyde in 0.067 M cacodylate buffer at pH 7.4, postfixed in osmium-collidine, and embedded in Epon 812. Thin sections were prepared on an LKB II Ultrotome, stained with a saturated solution of uranyl acetate and Reynolds' lead citrate,[12] and examined with a Philips 200 electron microscope. Thick sections (1.5 μ) were stained with 0.1% toluidine blue in 30% alcohol and examined by light microscopy.

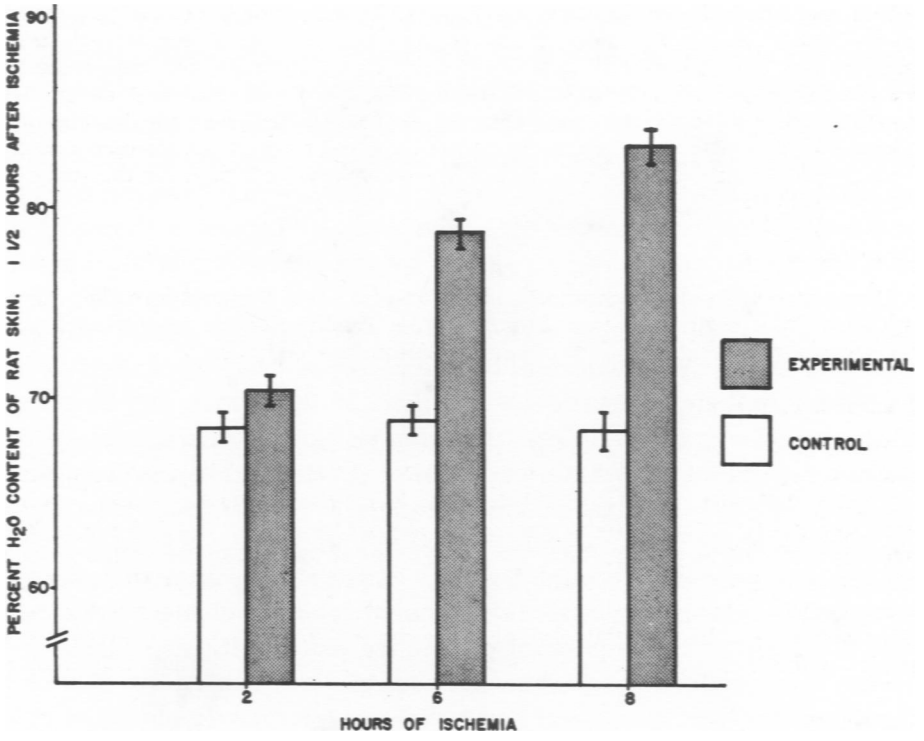
Terminology

Pressure area will refer to the zone of skin immediately underlying the metal rings of the clamp; *ischemic areas* will be those comprised within the rings. The term *vascular labeling* refers to the blackening of vessels after intravenous injection of carbon black; [13] the blackening is usually, but not always, indicative of vascular leakage.[14] The *time of ischemia* is the period during which the clamp remains applied; the period between release of the clamp and sacrifice of the animal is referred to for brevity as *reflow period* (even though no actual reflow may take place in the tissue which had been ischemic). Panniculus adiposus will be referred to as *adipose tissue* and panniculus carnosus as *striated muscle.*

Results

Rat Skin

Gross Observations. When carbon black was injected I.V. just before applying the clamp, the entire animal became dusky gray, then cleared very rapidly, except of course the area comprised within the rings of the clamp where the skin remained gray. When the clamp was released, the two discs of gray skin became pink immediately; at the same time, vascular labeling developed in the area of compression, but not in the central areas (except for occasional venules in the muscle and subcutaneum). Evans blue showed a similar pattern; there was immediate bluing of the pressure rings, but none in the ischemic areas, even after 8 hr. of is-



TEXT-FIG. 2. Skin edema after ischemic insults of increasing duration (all rats sacrificed 1½ hr. after removal of skin clamps). Water content of normal skin was 68.4 ± 0.9%; the increase after 2 hr. of ischemia was not significant (70.3 ± 0.9%); after 6 and 8 hr. it was highly significant ($p < 0.001$).

chemia, no matter how late the Evans blue was injected after release of the clamps. The ischemic areas always developed immediate "hyperemia" which lasted 10–15 min. and was often more intense when the ischemia had lasted 6–8 hr. One of the most striking results was the appearance of edema after ischemia, particularly when the latter had lasted 6 hr. or longer (Text-fig. 2); the edema reached a maximum 1–2 hr. after reflow, then receded slowly and disappeared completely in 24 hr. if the skin had been ischemic for no longer than 2–6 hr. Skin exposed to less than 6 hr. of ischemia rarely showed any palpable trace of a lesion at 24 hr., whereas 8 hr. of ischemia produced an induration with varying degree of cyanosis; after 24 hr. hair growth was absent or markedly diminished in all animals which otherwise showed active growth in depilated areas.

Light Microscopy. No significant changes were found in skins subjected to less than 2 hr. of ischemia. After this time minimal damage consisting of sporadic hyalinization and accentuation of cross-striations in

the fibers of striated muscle and some blebbing of the vascular wall in vessels in the adipose tissue were observed (Fig. 1). Reflow periods of 24 hr. showed some increase in the hyalinization of muscle fibers and occasional PMN infiltrate. In one animal this infiltrate was quite prominent. Mast cell stains demonstrated occasional degranulation in the muscle layer. The hypodermis, dermis, and epidermis appeared normal at this time. Ischemic periods of 4–6 hr. with 0–2 hr. reflow showed loss of fibers in the muscle, accompanied by considerable PMN infiltrate, edema, and necrosis, which also occurred in adipose tissue (Fig. 2); necrosis of muscle became more marked if the reflow period was extended to 24 hr., and edema had been reabsorbed to a large extent (Fig. 3). Vessels in these layers showed margination, diapedesis, and blebbing of the vascular wall. The skin from animals with 8 or more hr. of ischemia showed an almost complete loss of striated muscle, even in skin taken immediately after the clamp was released. Swelling and necrosis of fat cells, blebbing of the vascular wall, PMN diapedesis, and infiltration became very intense, although edema was partially reabsorbed after 24 hr. (Fig. 4). Congestion was also frequent. Dermal changes consisted only of edema and disruption of connective tissue, with some PMN infiltrate, but very few vessels showed margination or diapedesis. Necrosis of epithelial cells in the base of hair follicles and some vacuolation of epidermis usually were found after 8 hr. of ischemia.

In the pressure area, necrosis of vessels and other components of the skin were already seen after 2 hr. of clamping. A complete loss of striated muscle, PMN infiltrate, and carbon labeling of vessels were the most important changes of this area.

Rabbit Ear

Gross Observations

In the depilated rabbit ear, seen by transillumination, the vascular pattern is extremely clear. When the clamp is removed, the compressed area appears at first as a pale white ring, devoid of any visible vessel, but almost immediately the large central artery reappears as a distinct streak across the pale ring (Fig. 5a), no matter how long the clamp has been applied. In some animals a brief period of arterial spasm at the pressure point was followed by prolonged dilatation (Fig. 5b). After 8 hr. of compression, the large "marginal" vessels and all smaller vessels in the pressure area took up to 30 hr. to reopen; many remained narrowed even after 1 hr. of reflow. Animals allowed to survive for 24 hr. or 5 days showed complete recovery of the vascular pattern in all cases except one, in which thrombosis of the inner marginal vein developed. Two animals

subjected to 8 hr. of ischemia, and examined 5 days later, showed virtually complete recovery of circulation, with only slight epidermal excoriations along the pressure-ring area in one rabbit.

Vascular labeling developed according to the same pattern described for the rat: when carbon black was injected just prior to the release of the clamp, marked labeling occurred in the area of direct compression (Fig. 5b), with very little in the central areas (with the exception of one animal, in which the central areas did develop more extensive labeling, though still less than in the pressure area).

Light Microscopy

Although the number of rabbits studied is much smaller than the number of rats, the impression we gathered from specimens studied by light microscopy is that the tissue damage was considerably less, even after 8 hr. of ischemia (Fig. 6). Blebbing of the vascular wall and intravascular changes were absent, and in a few animals the only evidence of vascular injury was the presence of red blood cells in the interstitium. PMN infiltrate was virtually never seen, and only a slight swelling of the hypodermis, close to the cartilage layer, was seen in some skins after 6 or 8 hr. of ischemia. No significant differences were found between short reflow periods (30 min. to 1 hr.) and longer reflow periods (up to 24 hr.).

Electron Microscopy

Ultrastructural Changes of Vascular Wall. Since the changes were qualitatively the same in rats and rabbits, the two groups will be described together. One of the characteristic aspects of the ischemic damage was the focal nature of the lesions. Severely damaged vessels were often adjacent to morphologically normal ones. In general, the proportion of normal to damaged vessels increased with the duration of ischemia, but individual differences were marked, even after 8 hr. of ischemia. Also, one type of vascular damage did not necessarily imply or exclude any other. For this reason, the description of the lesions will be based on an arbitrary choice from the earliest signs of injury to what undoubtedly were advanced phases of necrosis.

Control skins, in general, were well preserved; some mild fixation artifacts, such as swollen mitochondria and some swollen endothelial cells, were found in approximately 2 of every 10 vessels in rat skins, and seldom in rabbit specimens.

In ischemic skins, the earliest evidence of endothelial damage was a diffuse swelling of individual endothelial cells; it was indistinguishable

from that seen in some control vessels, where presumably it represented a fixation artifact; however, after ischemia it was present in a much higher proportion of cells. It was characterized by a rarefaction of the cytoplasm, often confined to a single endothelial cell (Fig. 7), but in some small venules and capillaries it involved all cells (Fig. 8), with corresponding narrowing of the lumen. Myelin figures were often present in such vessels. Correlated with swelling was blebbing of the endothelial cell (Fig. 9), a phenomenon which sometimes led to almost complete obstruction of the lumen. In many vessels, these blebs became detached from the endothelium and were found free in the lumen (see below and Fig. 10); in several instances the blebs apparently had collapsed, giving rise to a "cauliflower" structure (Fig. 11). Another type of early endothelial change was the swelling of the perinuclear space; a characteristic feature of this lesion was that the two nuclear membranes remained attached at the level of the nuclear pores (Fig. 12). Condensation and margination of nuclear chromatin in endothelial cells were constant after 6 or more hr. of ischemia (Fig. 12). In pericytes, the most frequent changes were swelling of endothelial reticulum (ER) and of the perinuclear spaces (Fig. 13); these changes paralleled those of adjacent endothelium.

Notably rare were endothelial gaps of the type induced by histamine.[13,14] It must be noted, however, that most of our ultrathin sections were taken from the dermis, where labeled vessels were seen only occasionally by light microscopy and then showed very small amounts of intramural carbon. The majority of leaking vessels were found in sections from deeper areas of the skin—i.e., adipose tissue and striated muscle, and usually were associated with other evidence of severe injury, such as extravascular fibrin (Fig. 14). Lymphatic endothelium also showed considerable injury after 6–8 hr. of ischemia (Fig. 15): dilatation, endothelial gaps, swollen ER, and clumping of cytoplasmic contents.

In general, the vessels of rabbit ear skin (as seen by electron microscopy) responded somewhat differently from those of rat skin in two respects: small, carbon-labeled leaks were more frequent, whereas signs of endothelial injury (swelling, blebbing) were less.

Intravascular Changes. Significant changes were found only in skins which had suffered 8 hr. of ischemia, and then only in the skin of the rat. Spherical bodies in the lumen, surrounded by a tenuous membrane and containing material less dense than cytoplasm, similar to the "blebs" described by Chiang *et al.*,[4] were found occasionally, close but not attached to endothelium (Fig. 10). Plugging of small vessels by white or red blood cells (Fig. 16 and 17) was also encountered frequently. Plate-

let thrombi were found only in areas of severe tissue damage, often together with masses of fibrin (Fig. 16).

Extravascular Changes. Large spaces appeared in the interstitium as a result of edema, and some electron micrographs suggested that swelling could compress or distort small vessels. There was some dissociation of collagen bundles, particularly around blood vessels; fibrin and free red cells were also found. Cellular swelling and necrosis, particularly of fibroblasts, and degranulation of mast cells were roughly proportional to the time of ischemia. Fat cells seemed to "explode," and the resulting droplets of free fat were either trapped in the interstitial spaces or taken up by histiocytes.

Epithelial cells of hair follicles showed increasing vacuolation as the time of ischemia was prolonged; after 8 hr. virtually every cell in the hair follicle showed marginated and clumped chromatin.

Discussion

Resistance of Skin to Ischemia

From the results of various clinical and experimental studies on the survival of skin homografts and metabolism of connective tissue, it could have been anticipated that the skin would survive relatively long periods of ischemia without irreversible damage.[6,7] This was the case: In both rat and rabbit experiments it was found that the epidermis, dermis and hair follicles showed little injury after 2–6 hr. of ischemia, the only sign of damage being retarded hair growth of the area, particularly in the rat. Ischemia lasting more than 8 hr. invariably caused necrosis and sloughing of tissue within 5 days of the ischemic insult in the rat. This is remarkably similar to the figure given recently by Selye[10] despite several differences in the experimental technique. Selye raised a fold of skin from the back of the rat (just as we did), then constricted its base with an umbilical clamp. In his normal animals necrosis ensued with 9 hr. of ischemia; pretreatment with large doses of Thorazine, various forms of stress, epinephrine, or noreprinephrine increased the resistance of the skin to this period of ischemia, although some injury was found on the fourth day when animals were sacrificed. The maximum tolerance found in our experiments corresponds approximately to that of Selye's normal animals, despite the administration of Thorazine and the mild stress of our restraining board (both, according to Selye's findings, could have prolonged the tolerance in our rats*) and despite the fact

* The dose of Thorazine given our animals was 1 mg./100 gm. body weight, slightly less than that given by Selye; the restraining board employed by Selye is, of course, much more stressful than the one used in our experiments.

that our constricting device was probably less traumatic to the tissues than the umbilical clamp.

One of the most striking observations was the greater sensitivity of the intrinsic muscle of rat skin, as compared to the dermis or epidermis. This observation in itself is not surprising, since it is known that striated muscle degenerates rapidly when the blood supply is cut off for more than 2 hr.[15] Studies on the pathogenesis of decubitus ulcers have shown that the striated muscle underlying the area degenerates and becomes infiltrated with PMN's long before any dermal changes appear.[8,9] Additional proof of a higher resistance of epidermis and dermis to ischemia is found in the results of rabbit ear ischemia. The skin of the rabbit ear has no fat or muscle in the hypodermis; thus, early necrosis of these layers did not interfere with the survival of the skin, and very little dermal injury was seen even after 8 hr. of ischemia. In animals kept for 5–6 days after 8 hr. of local ischemia, there was an almost complete absence of visible damage. In the field of skin transplantation it has been known for some time that pieces of skin which include layers of fat or muscle have a much lower survival rate than so-called split-thickness grafts.[6,16] The greater survival ability of the epidermis may be related perhaps to diffusion of oxygen from the surface during the early hours of ischemia, whereas hair follicles, the activity of which is inhibited in our experiments, depend entirely on O_2 from the underlying capillary circulation.[17]

Problem of Reflow after Ischemia

When ischemia is produced by compression of tissues with devices such as the tourniquet or the pressure rings of our experiments, the problem of reflow after ischemia is affected by two sets of variables: vascular changes in the compressed tissue and vascular changes in the ischemic tissue.

The compressed tissue is obviously of great importance, since it represents the gateway through which the ischemic skin will receive all its reflow. In our experiments, the pressure was measured and maintained at a level just adequate to prevent arterial flow, and was presumably less injurious than that of a rubber tourniquet such as is applied on rat limb.[8,18] Despite this precaution, vascular injury in this area was obvious; vascular labeling with carbon and bluing occurred almost exclusively here, both in the rat and rabbit models. What actually happens as a direct result of pressure is demonstrated beautifully in the rabbit ear, which is transparent enough for this study: for 10–30 min. after removal of the rings, depending on the time of ischemia, the pressure area remains bloodless, except for the larger arteries. The vessel

walls remained pressed together; only the larger arterial vessels can be forced open, thanks to a higher intraluminal pressure. Gradually, some of the larger veins begin to open up; and finally, the capillary network is also filled. This is the overall pattern. Vasomotor changes also take place, particularly the well-known arterial dilatation after ischemia (Fig. 5). The effect of this sequence on the reflow in the central (post-ischemic) areas can only be inferred in qualitative terms: first, there should be an arterial inflow only, with greater filling of all vessels, then some degree of passive congestion, as the effluent veins gradually resume function. Whether this hyperemia ultimately may be defined as active, passive, or both in sequence must await quantitative studies of blood flow.

A characteristic event after ischemia in other organs is the "no-reflow" phenomenon, particularly obvious in the brain,[2,3] but clearly demonstrable also in the adrenal[19] and the striated muscle of the rat hind limb.[18] In all these tissues after ischemia, certain areas remain temporarily or permanently incapable of resuming blood flow; this is demonstrated best by perfusing the organ with carbon black after ischemia. We attempted to perfuse the rat skin after ischemia, but both control and postischemic skins showed incomplete filling patterns of vessels, the conclusion being that in our system the vessels of normal skin could not be uniformly filled before the animal died. The overall impression was that the skin is much less prone than other organs to develop no reflow after ischemia. Several other observations point to the same conclusion. When the skin was made ischemic with carbon in its blood vessels, the carbon (as judged by eyesight) was washed away immediately as pressure was released. Furthermore, edema developed rapidly, and was then reabsorbed within 24 hr. (unless ischemia had lasted longer than 6 hr., both changes suggesting a fair degree of circulation. When ischemia of the rat hind limb is produced with a tourniquet, areas of no reflow can be demonstrated in the muscular masses, but the corresponding skin remains well perfused.[18] The reason for this better ability of the skin to preserve its circulation may be that it has no true parenchyma between its blood vessels: parenchymal cells play a major role in causing vascular disturbances after ischemia.[18,20] The mechanisms remain largely conjectural, but at least two appear very likely: swelling parenchymal cells remove fluid from static blood vessels,[4,18] and massive necrosis of parenchymal tissue (such as muscle or fat in our rat skin model) may affect, secondarily, the pertinent blood vessels.

Apart from the changes in the pressure area, two other variables affect the reflow in the skin after ischemia: (1) changes in the vessels

themselves, to be considered shortly, and (2) local arterial blood pressure. It is clearly shown, in the rabbit ear model, that part of the obstacle to reflow is purely mechanical; a collapse of the vascular walls. This and other obstacles to be described further may be overcome if arterial pressure is adequate. In our rabbit ear model, a major artery led directly into the experimental area, and this may explain, in part, the greater resistance of the rabbit ear to ischemia.

Pathogenesis of Skin Edema after Ischemia

Postischemic edema was a constant occurrence (Text-fig. 2). Initially, we interpreted it as a result of vascular leakage, caused by mediators liberated from injured mast cells. Electron microscopic studies did not bear out this interpretation: endothelial gaps were very few and even those did not have the characteristic features of histamine-type gaps. It has been shown recently that the mediators of the histamine group induced, in the membrane of endothelial nuclei, typical tight folds ("pinches") that are best explained as a result of cellular contraction.[21] Others have shown, by fluorescence methods, that endothelium contains material with the characteristics of actomyosin.[22] The combined evidence strongly suggests that histamine-type mediators induce at least some endothelial cells to contract and thus to produce intercellular gaps. The absence of "contracted" endothelial cells in our material was at first surprising; yet it may be satisfactorily explained on the very basis of endothelial contractility. It is a well-known fact that preparations of smooth muscle fail rapidly in vitro if the bathing fluid is not aerated; if glucose is removed, the contractile response of guinea pig ileum is affected within a few minutes.[23] If endothelium is postulated to have contractile properties similar to those of smooth muscle, then it should also fail to respond to mediators in ischemic tissues. Another experimental model lends support to this conception: in the rabbit ear, the Arthus phenomenon leads to arterial contraction; but if the ear is submitted to ischemia for 1 hr. prior to injection of the immune serum, the arterial spasm is delayed.[24]

Two other factors must be taken into account in explaining the rare occurrence of leakage in postischemic skin: (1) Poor circulation. There are several reasons to believe (see further) that the circulation is somewhat impaired after ischemia (the active hyperemia described in physiology refers to very short bouts of ischemia [25]). If the circulation is poor, vascular labeling, which depends on flow,[26] will also be impaired. Rabbit ear lesions, with a better circulation than rat skin, showed more leakage than comparable rat lesions. (2) The vessels examined by

electron microscopy were mainly dermal—i.e., capillaries, which are particularly resistant to histamine-type mediators and possibly also to other types of vascular injury.[14] Having ruled out histamine-type vascular leakage as a major factor in postischemic edema, it should be noted that other types of endothelial gaps were also infrequent: destruction of the endothelial cells of the "direct injury" type as an immediate consequence of ischemia was found consistently only in the pressure area.[27]

Other factors that undoubtedly contribute to the pathogenesis of postischemic edema of the skin are: (1) venous congestion, due to the delayed reopening of effluent veins, as described above; (2) lymphatic blockage, due to a similar mechanism (a huge dilatation of lymphatics was often observed); and (3) osmotic forces. The presence of circulating blood (in the skin) is not essential for the appearance of edema; this is well demonstrated by skin grafts, which have some resemblance with our experimental models of ischemic skin. The clinical observation that skin grafts accumulate fluid during the first hours after transplantation was first made by Garré in 1889.[28] More recent studies by Converse *et al.*[29] and Clemmesen [6,16] show that split-skin autografts increase in weight during the first 48 hr., which can only be explained by assuming that fluid is being supplied by the graft bed.* The sequence of events suggested by Hynes [30] and accepted by Clemmesen would be the following: fluid oozes "into the capillaries" of the graft, and since these vessels have become hypoxic and thus leak, plasma escapes into the tissues. This explanation is scarcely defendable. It is much more likely that metabolites accumulate in the anoxic skin, with resulting movement of the fluid into the graft from the surrounding tissues—a form of edema without circulation. A similar, but not identical situation occurs when fragments of parenchymal organs are allowed to die within the living body—a rapid increase in weight occurs, due to the uptake of water in the absence of circulation.[31]

In apparent contrast with the very "general" nature of the ischemic insult, the vascular damage was characteristically, and unaccountably, focal; often it was limited to single cells. This particular behavior of the endothelial lining has been noticed in several studies concerning direct and indirect vascular injury.[32,33] The selective and progressive necrosis of endothelial cells after ischemia has been reported by Reinecke *et al.*,[34] who studied the capillaries of the cat retina. This study showed a progressive loss of endothelial cells when retinal vessels were subjected

* In grafted human skin the first signs of circulation due to "inosculation" appear after 18 hr.[29]

to more than 1½ hr. of total ischemia, so that 35 days after the ischemic insult only 25% of endothelial cells and a network of acellular strands remained. It was not clear whether these strands were patent, since the mural cells (pericytes) remained intact.

In our experiments the first ultrastructural evidence of endothelial damage appeared 3 hr. after an ischemic period of 2 hr. A clearcut sequence of changes could not be established, but swelling of the ER (Fig. 12), swelling of the whole cell (Fig. 8), and dilatation of the perinuclear cisternae (Fig. 13) seemed to be the earliest events. A more localized form of swelling was represented by the cellular blebs, blister-like protrusions of the endothelial cells which have been described as a result of brain ischemia,[3] limb ischemia,[18] and other types of tissue injury.[32,35,36] The mechanism of this blebbing is unexplained as yet; it appears to be a general manifestation of cellular injury. The blebs never reached a size large enough to obliterate the lumen; however, they must constitute a measurable obstacle to the circulation.

Other intravascular obstacles, including thrombi, were rare. A few capillaries appeared plugged by red or white blood cells, but whether this represented a true obstruction cannot be decided on a morphologic basis. Suggestive evidence for plugging is in the mere fact that too many "occluding" leukocytes were found; under normal circumstances, the chance of encountering a leukocyte in a capillary is small. It is possible that the postischemic endothelium may have become stickier to leukocytes.[37]

Summary and Conclusions

1. A technique is described for producing ischemia of animal skin, using a specially constructed clamp which permits occlusion of the circulation in a ring-shaped area without excessive injury to underlying tissue.

2. Epidermis and dermis of rat and rabbit, in the models here described, survive periods of ischemia as long as 6 hr. Long-term survival of rat skins after 8 hr. of ischemia shows eventual sloughing and necrosis of the area, whereas rabbit ear skin shows no significant change, even after 5–6 days.

3. Fat cells and striated muscle are much more sensitive to ischemia; when they are components of the skin, they complicate the picture of postischemic necrosis, and therefore decrease the overall resistance of the skin to ischemia.

4. Vascular injury of a focal nature is so severe after 8 hr. (in rat

skin) that it appears to be a critical factor in determining the ability of the skin to survive ischemia.

5. In our models of skin ischemia, damage to the vessels of the pressure areas is partly responsible for poor reflow in the ischemic areas. A similar situation probably prevails after tourniquet ischemia.

6. Despite extensive mast cell damage, vascular leakage of the type induced by chemical mediators appeared to be, at best, of secondary importance in the pathogenesis of postischemic edema. In view of the evidence that endothelium is contractile, it is suggested instead that ischemia abolishes the capacity of blood vessels to respond to such mediators.

7. Skin edema after ischemia is the result of several factors: reflow of arterial blood with delayed outflow (due to pinching of the veins); pinching of the lymphatics; some vascular leakage; and probably also hyperosmosis due to accumulation of catabolites (the latter mechanism of edema would function also in the absence of reflow—i.e., in skin grafts).

8. Prior to the failure of the circulation, a number of vascular changes were found; the ultimate failure of flow may be considered the result of all these limited, focal changes.

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We are indebted to Miss Juliette Spurgeon for skillful technical assistance, and to Mr. Eduardo Garriga for the photographic prints.

[*Illustrations follow*]

Legends for Figures

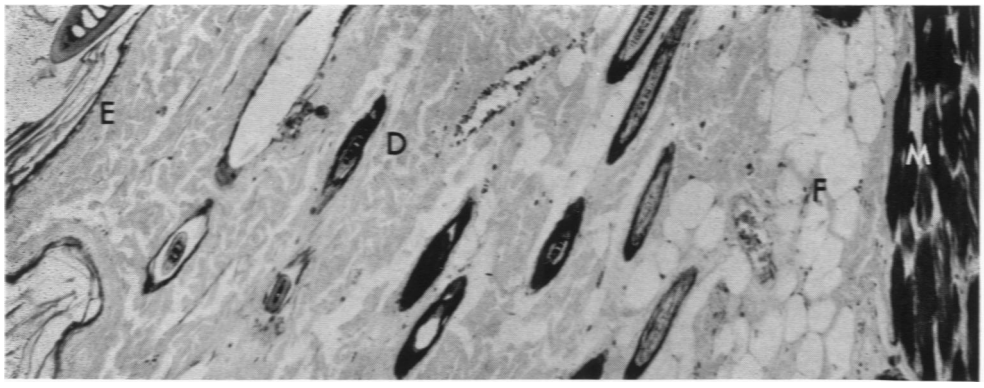
Figures 1–4 are light micrographs demonstrating effect of ischemia on rat skin. ×80.

Fig. 1. After 2 hr. of ischemia and 3 hr. reflow. Dermis appears normal except for a vein *(center)* with margination and some diapedesis. Adipose tissue *(F)* and intrinsic muscle *(M)* appear normal at this time. Epidermis *(E)*.

Fig. 2. After 6 hr. of ischemia and 1½ hr. reflow. Edema in muscle and adipose tissue *(F)* displaces dermis and hair follicles toward epidermal surface. Note PMN infiltrate in fat layer *(brackets)*, edema around hair follicles, and incipient hyalinization of muscle layers *(arrow)*.

Fig. 3. After 6 hr. of ischemia and 24 hr. reflow. Edema in adipose layer *(arrow)* has been largely reabsorbed; there is necrosis of muscle fibers *(M)*.

Fig. 4. After 8 hr. of ischemia and 24 hr. reflow. Edema is receding; collagen fibers in dermis remain dissociated (compare with Fig. 2). Adipose layer appears well preserved, but all muscle fibers *(M)* are abnormal.



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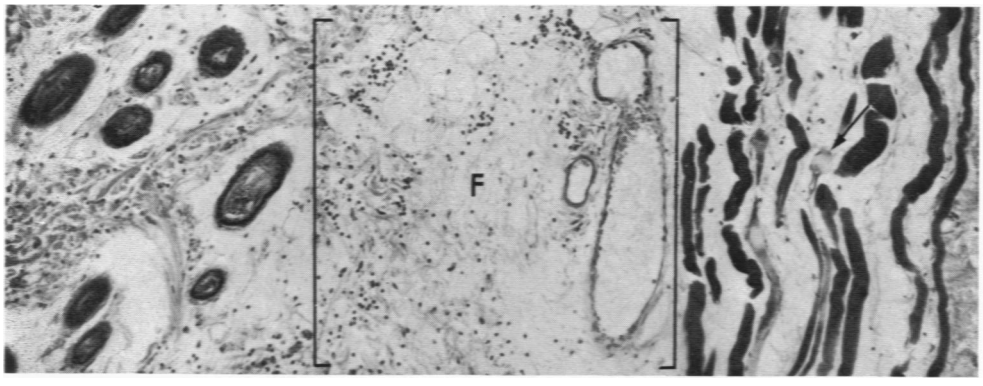

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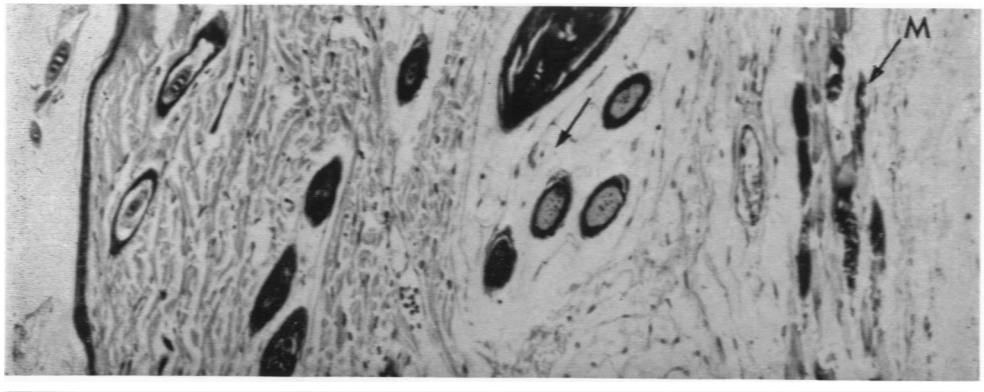

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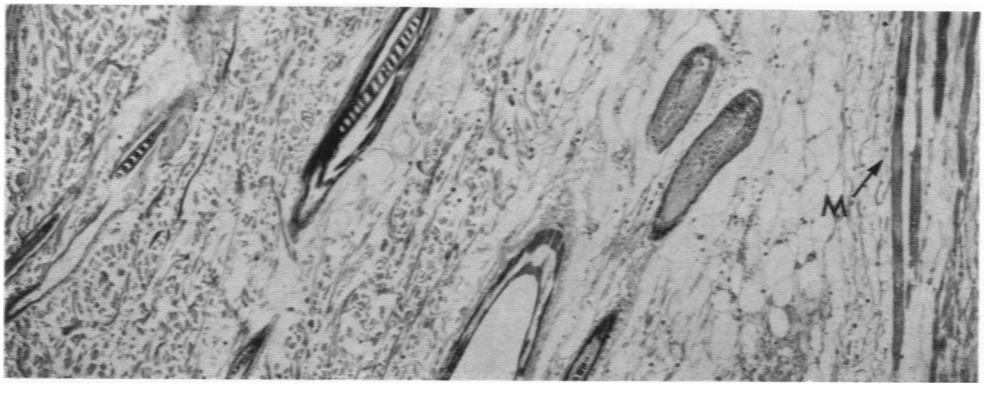

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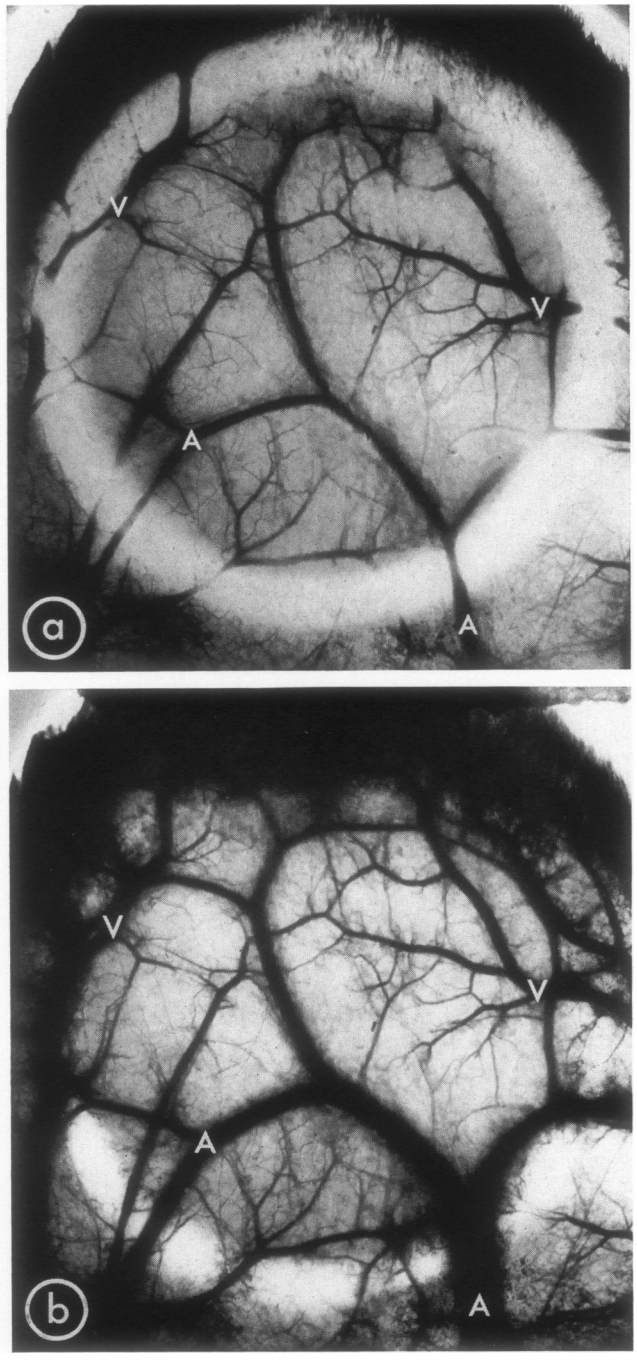

Fig. 5. a. Living rabbit ear after 6 hr. of ischemia, and 1 min. after release of clamp and I.V. injection of carbon. Medial artery and collaterals (A) have already reopened, although not completely. Major veins (V) are still collapsed. Note complete lack of capillary circulation in the pressure area. × 2. **b.** Same area 8 min. later. All large arteries and veins are markedly dilated. Capillary circulation in pressure area has resumed, especially at the top. × 2.

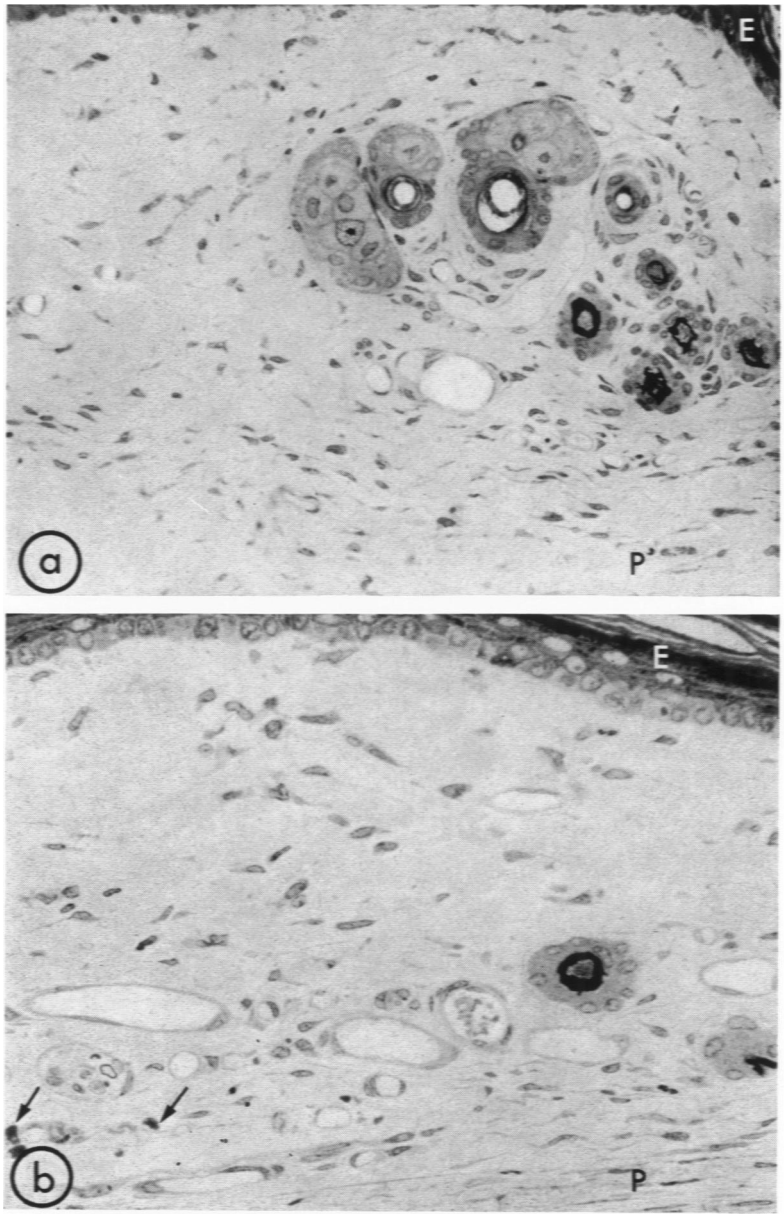

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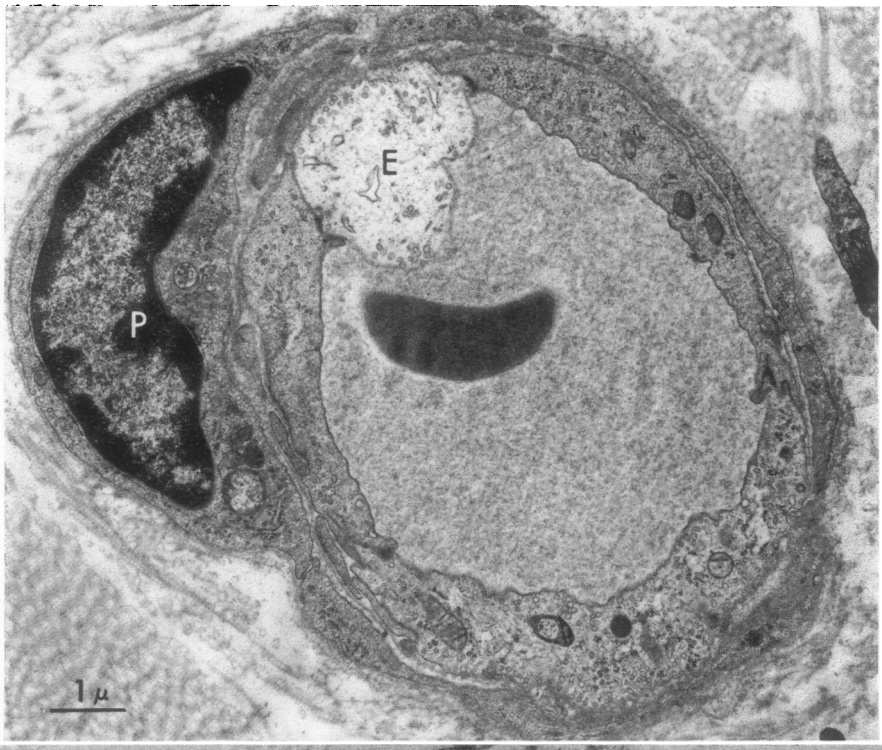
Fig. 6. Illustration of the relative resistance of rabbit skin to ischemia. Epidermis *(E)*; perichondrion *(P)*. **a.** Normal ear skin. Note absence of fat or muscle in hypodermis. **b.** Ear skin after 6 hr. of ischemia and 24 hr. reflow. No trace of injury except a few congested capillaries in hypodermis *(arrows)*. "Thick" Epon sections. × 230.



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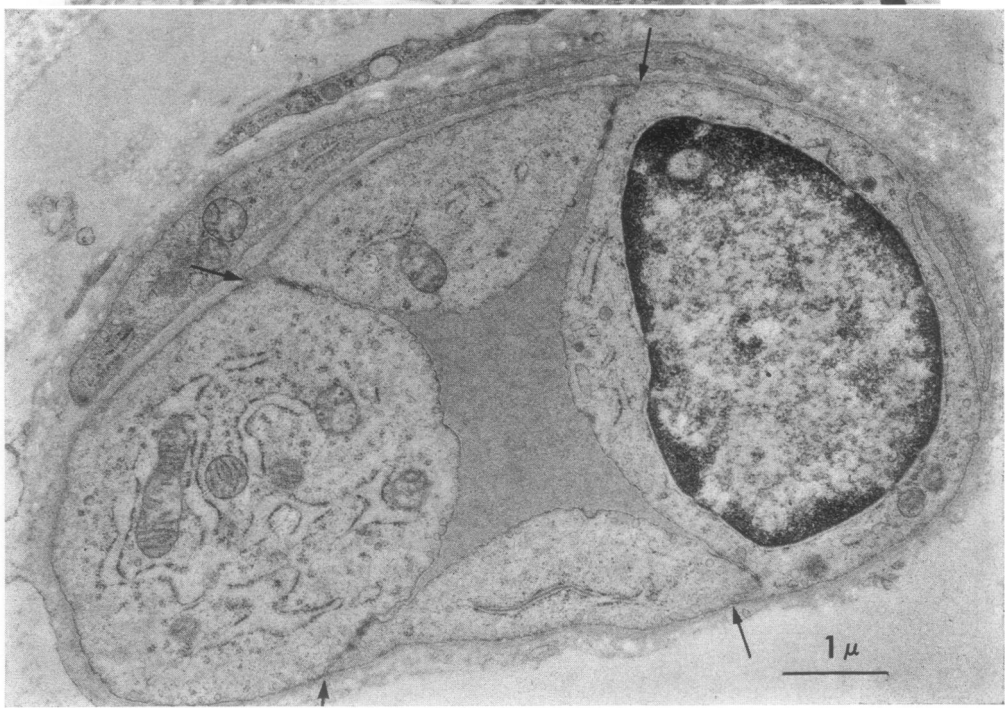

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Fig. 7. Small venule from rat dermis after 2 hr. of ischemia and 3 hr. reflow, illustrating one of the earliest forms of vascular injury: swelling of a single endothelial cell *(E)*. Other endothelial cells and pericyte *(P)* appear normal.

Fig. 8. Capillary from hypodermis of rabbit ear, after 8 hr. of ischemia and 24 hr. reflow. Swelling of all endothelial cells has greatly reduced the lumen. Arrows point to endothelial junctions.

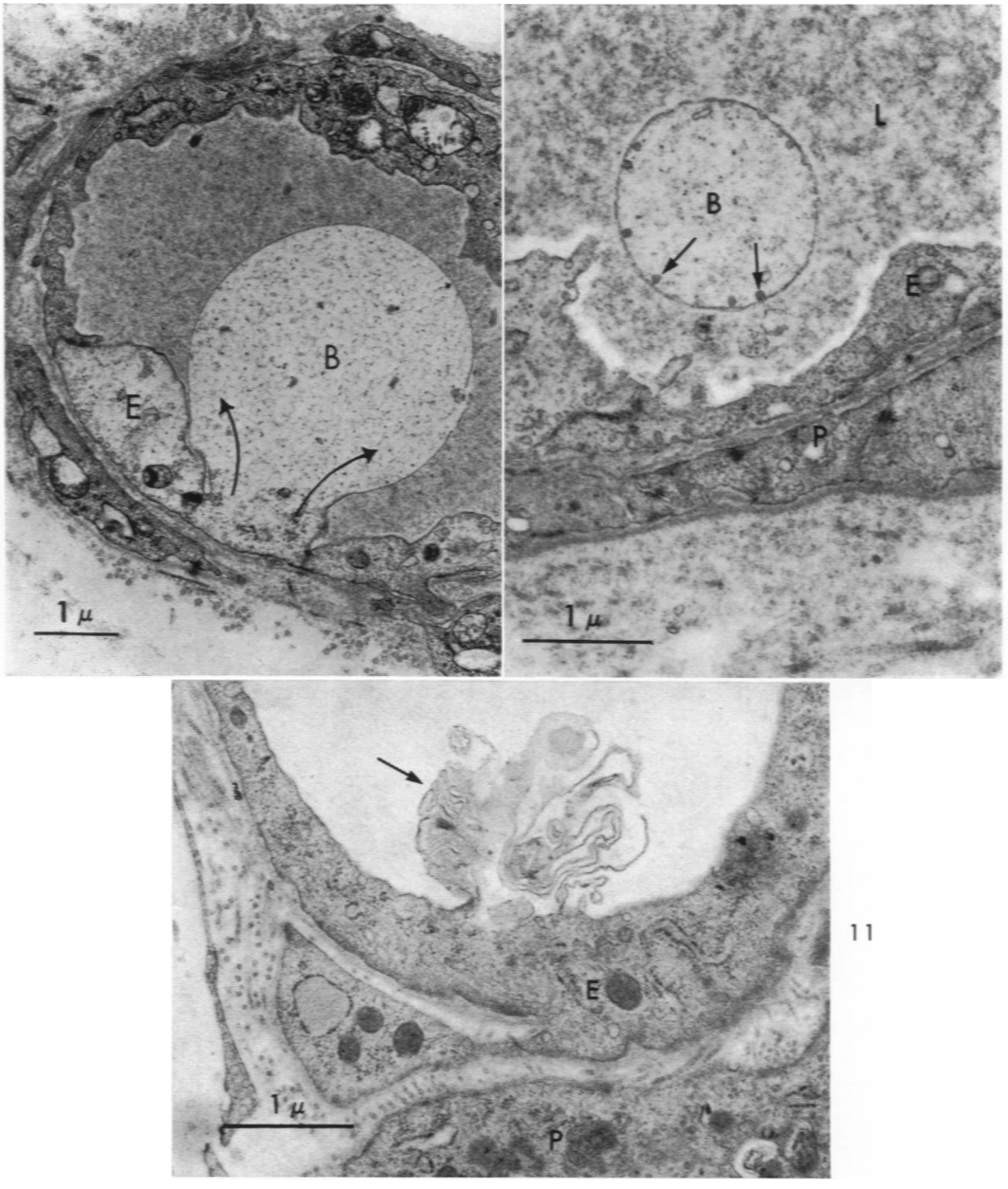


Fig. 9. Detail of a venule from rat dermis after 9½ hr. of ischemia and 23 hr. of reflow. Swelling of endothelial cell *(E)* with formation of a "blister" *(B)*, which is obstructing the lumen almost completely. Swollen ER and mitochondria appear in other endothelial cells.

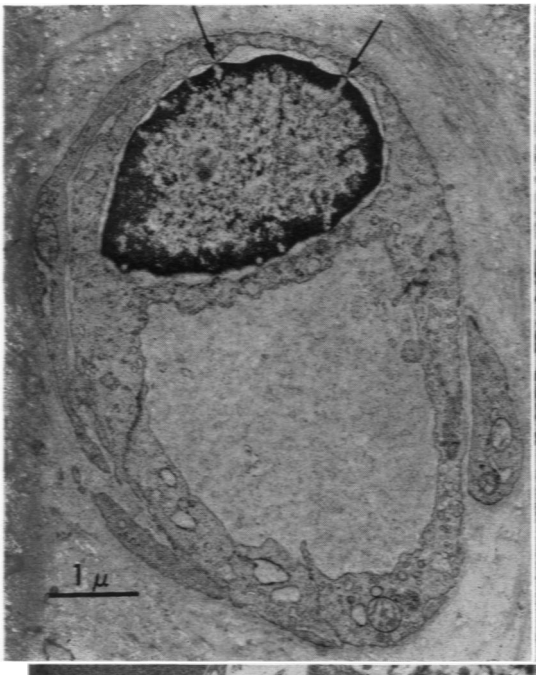
Fig. 10. Detail from vessel of rat skin after 8 hr. of ischemia and 24 hr. reflow. In the lumen *(L)* is a spherical structure *(B)* containing small vesicles *(arrows)* similar to those of normal endothelium. This structure could represent a grazing section of a bleb as illustrated in Fig. 9, or a free-floating structure of similar origin. Endothelium *(E)*; pericyte *(P)*.

Fig. 11. Detail from a venule of rabbit hypodermis after 6 hr. of ischemia and 24 hr. reflow. Membranous structure protruding into lumen *(arrow)* may result from collapse of a "blister" such as illustrated in Fig. 9. The rest of the endothelium *(E)* and adjacent pericyte *(P)* look normal.

Fig. 12. Capillary from rat skin after 2 hr. of ischemia and 3 hr. reflow. Endothelial cells show slight diffuse swelling and distended endoplasmic reticulum. Note incipient widening of perinuclear spaces; nuclear membranes remain attached at level of the pores *(arrows)*.

Fig. 13. Rat skin, after 6 hr. of ischemia and 18 hr. reflow. Severe injury in a venule, with grossly distended perinuclear spaces *(Ps)* in the pericyte *(P)* and distended endoplasmic reticulum in endothelium *(E)*.

Fig. 14. Severe injury in a venule from muscle layer of rat skin, after 8 hr. of ischemia and 1½ hr. reflow. Endothelium is very thin and partially disrupted *(arrows)*. Note platelet thrombus *(Pt)*. Fibrin *(F)*, red blood cells *(R)*, and platelets are lodged between and behind endothelial cells, as well as in the interstitium.



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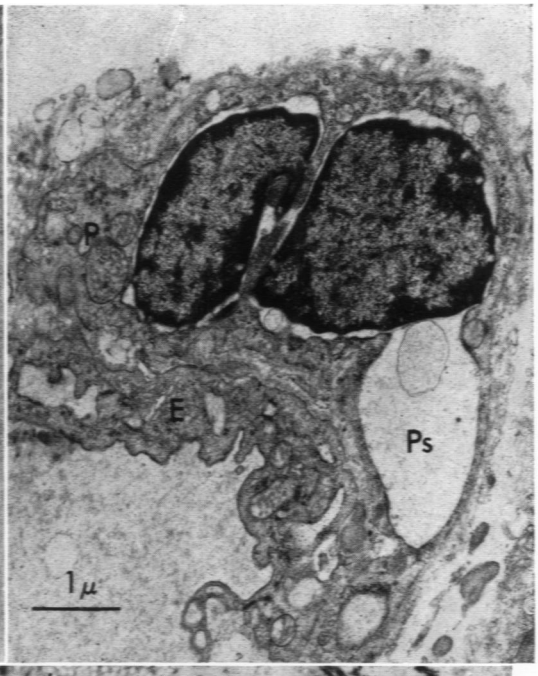

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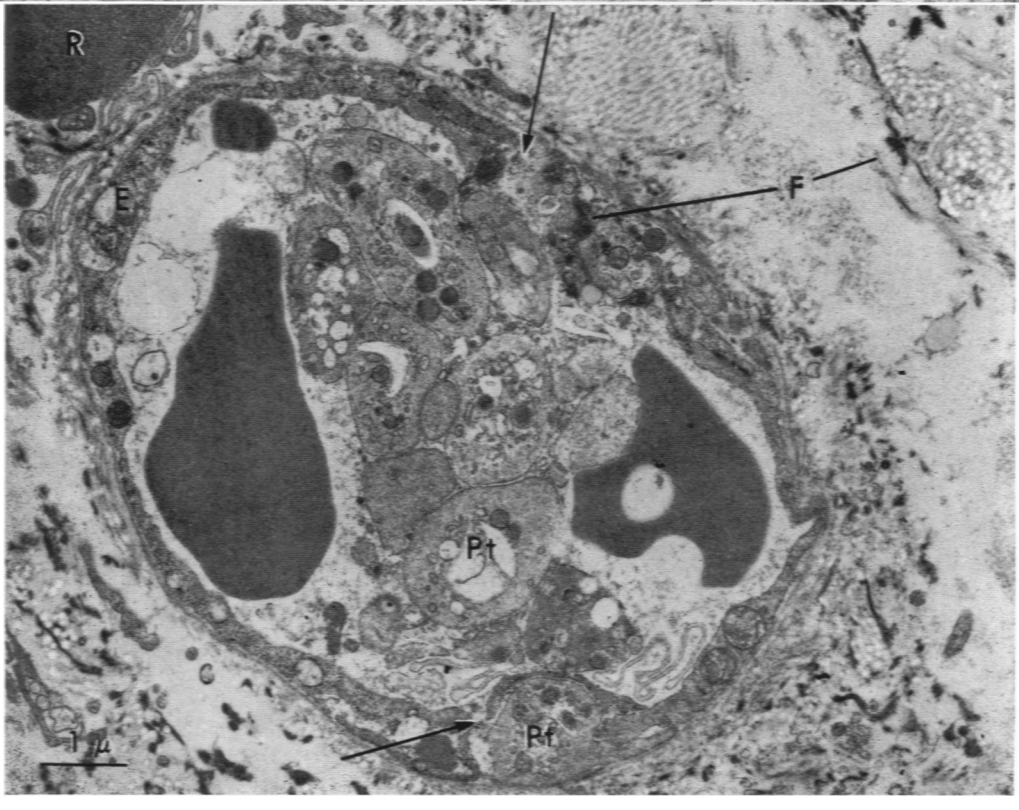

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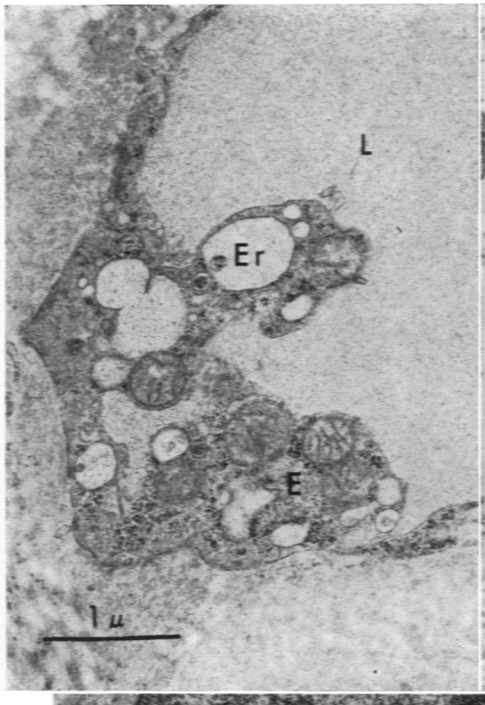
Fig. 15. Injured lymphatic endothelium *(E)* in rabbit ear skin, after 8 hr. of ischemia and 24 hr. reflow. Note swollen endoplasmic reticulum *(Er)*. Lumen *(L)*.

Fig. 16. Small capillary from rat dermis after 8 hr. of ischemia and 1½ hr. reflow, from an area with extensive tissue injury. Example of plugging by a white blood cell, probably a lymphocyte *(L)*. Endothelium *(E)* is abnormal (note swollen perinuclear spaces and ER). Arrow points to parts of extravasated red blood cells and platelets.

Fig. 17. Another instance of probable plugging by white blood cells, this time a polymorphonuclear leukocyte *(PMN)*. Endothelium *(E)* is slightly swollen. Lumen *(L)*. Hypodermis of rabbit ear skin, after 8 hr. of ischemia and 24 hr. reflow. Pericyte *(P)*.



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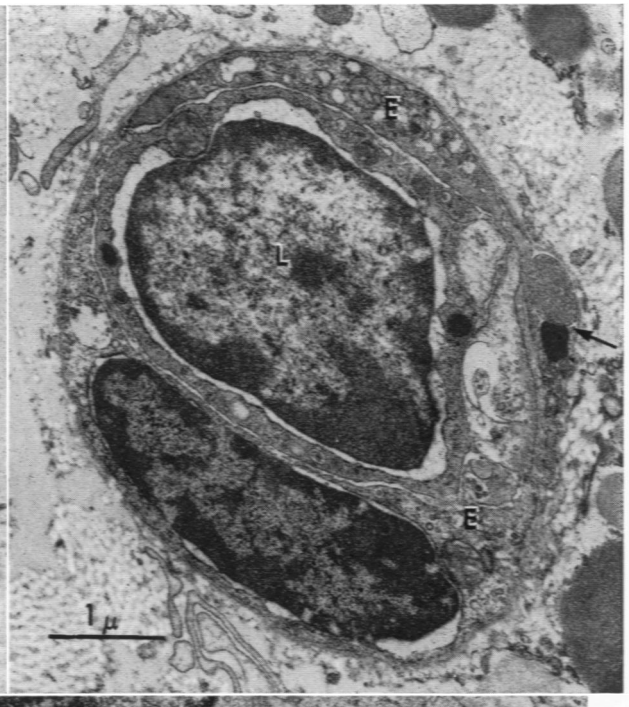

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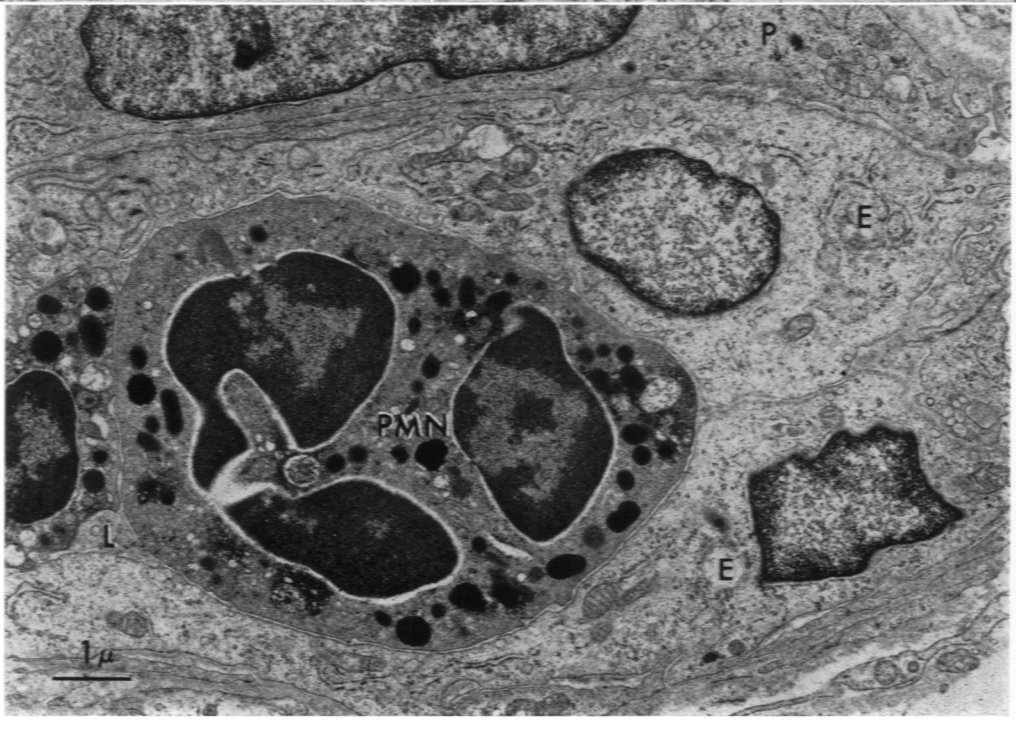

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