

## BRIEF COMMUNICATION

### A Model of Regional Microvascular Ischemia in Intact Skin

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#### INTRODUCTION

The advantages of using the mouse ear to study microvascular responses was recognized by North and Sanders (1958) who used it to study collateral circulation development following arteriolar ablation. Boykin and co-workers (1980) exploited the enhanced transparency of the homozygous hairless (h/h) mouse to study burn-induced microcirculatory changes which contribute to ischemia of the burn wound. Eriksson and co-workers (1980) underscored the use of the h/h mouse ear as a simple, inexpensive, and reproducible model for microcirculatory studies of intact mammalian skin. It has been used to study frost bite injury effects (Bourne *et al.*, 1986), the effects of superoxide dismutase following global ischemia (Barker *et al.*, 1987), and delayed healing processes associated with skin wounds (Bonder *et al.*, 1988) and to compare flow features in normal paired capillaries (Mayrovitz and Moore, 1989).

The microvascular response to skin ischemia is of considerable clinical interest and has been studied by intravital microscopy mainly in skinfold (Sack *et al.*, 1987) and skin flap models (Rosen *et al.*, 1985; Marzella *et al.*, 1988). Other studies have used the rabbit ear chamber (Zarem *et al.*, 1982) and the h/h mouse ear for the study of global ischemic effects (Barker *et al.*, 1987). When a tissue is made globally ischemic, one loses the advantage of being able to compare ischemic and nonischemic vessel responses in the same tissue. It is, thus, useful to have a method of producing selective regional ischemia which provides ischemic and nonischemic control regions.

When it is of interest to study pressure-induced ischemia, regional ischemia can be produced by compression of a region of tissue with a region outside this region serving as control. Romanus and co-workers used such a strategy on the hamster cheek pouch to study the microvascular responses to compressional ischemia (Romanus *et al.*, 1977; Romanus and Haljamae, 1978). Unfortunately, when using such methods, it is difficult to separate the effects due to compression forces from those due to the ischemia per se. Therefore, it would be of considerable use to have a noncompressional method of producing the regional microvascular ischemia using arteriolar ligation.

## METHODS

*Initial Procedures*

Adult homozygous hairless mice (SKH-1; Charles River Laboratories), 2–3 months of age, weighing between 35 and 40 g were anesthetized with pentobarbital sodium administered intraperitoneally (6 mg/100 g). After anesthetic induction the animal is placed under a surgical microscope (Zeiss OPMI6-S) and a 25-gauge minicath (Abbott Laboratories) is inserted in the anterior intraperitoneal cavity for the administration of maintenance anesthesia. The advantage of the indwelling catheter placement is the ease of supplemental anesthetic administration while recording data at high power. The minicath is secured with suture ligatures to the external abdominal wall to prevent inadvertent movement of the indwelling needle and thus, eliminating possible damage to internal abdominal structures. The animal is then wrapped in a 4 × 4-in. cotton gauze pad to prevent convective heat loss and placed in the supine position on an observation board which supports the animal's body with the ear positioned on an attached glass slide for microscopic viewing of the ear microcirculation (Fig. 1). Gross assessment of the vascular anatomy of the ear provides the basis for selection of the risk and nonrisk zones. Based on the vascular supply to the distal regions, they are classified as risk (RZ) or nonrisk (NRZ) zones. The RZ are those tissue regions which will be affected by subsequent arterial ligations and the NRZ are those that will not be significantly affected, and thus, are control regions. The anterior ear vasculature is usually more densely infiltrated with feeding arteries and collaterals than the posterior, thereby providing a greater sampling selection of capillary loops, yet also being the more difficult area in which to effect complete flow stasis. The gross ear vasculature is examined *in toto* and, based on the specific vascular distribution, a decision made as to RZ and NRZ. The ear is carefully positioned with the dorsal surface gently flattened against the slide, using paraffin oil on both the dorsal and ventral surfaces of the ear prior to placement of a No. 1 thickness micro-coverslip. This provides a flat surface area for investigation of the microvasculature. Temperatures are monitored using two thermistor probes (Bailey Instruments, Model BAT-8), one inserted rectally for monitoring core temperature, and another positioned in the external meatus of the ear. Temperatures are maintained using a heat lamp mounted on the micro-

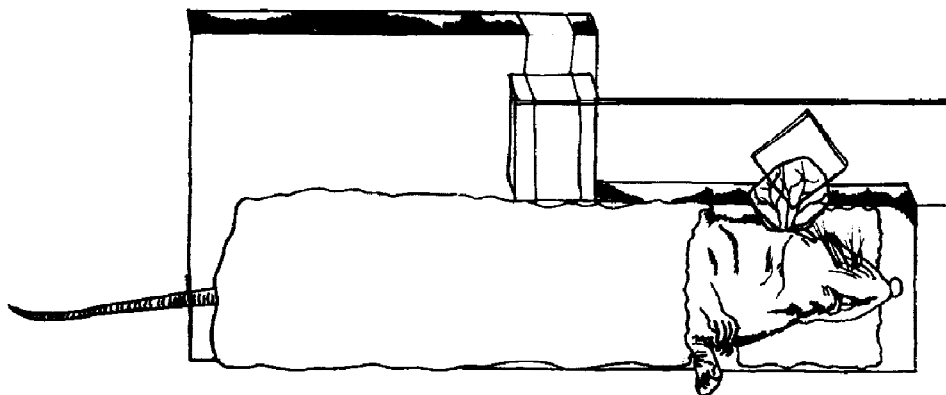


FIG. 1. Arrangement for observing the intact skin microvasculature of the hairless mouse ear.

scope; the core temperature is maintained between 32 and 35° and ear temperature between 28 and 31°. Heart rate is monitored using a Medsonics Photopulse Adaptor (Model PA 13) with the cuff attached to the proximal portion of the tail. This is in turn connected to a polygraph chart recorder (Grass RPS 7C8A).

### *Baseline Data*

Further investigation is conducted using a Leitz Laborlux microscope (12HL) fitted with a trinocular zoom magnifier which is coupled to a low light level TV camera (MTI 65) and associated video recording apparatus. Under transillumination, the ear microvasculature is examined using long-working distance air objectives; low power 10× for location purposes and 43× for capillary loop data acquisition. The corresponding total effective magnifications referred to the video monitors are 100× and 430×, respectively. Data obtained in this fashion are analyzed offline to determine capillary diameter, blood velocity, and other parameters. This method of recording also provides the reference points for subsequent relocation and identification of the capillary loops under investigation following occlusion and reperfusion preparations.

### *Regional Ischemia*

To effect regional ischemia, the animal is placed under the surgical microscope. Using microsurgical techniques the central artery, lateral artery (either anterior or posterior), and primary interanastomosing branches are ligated with 7-0 Vicryl suture at the mid-proximal level of the ear as shown in Fig. 2. A small piece of 2-0 cotton suture is secured between the skin and knot to prevent tissue damage or tearing. Initial occlusion effects are ascertained visually under the surgical microscope by the ischemic discoloration and vascular engorgement of the RZ as compared to the NRZ. A relatively prominent "border zone" area of compensatory collateral filling can be seen between the two affected regions. After ligature occlusion, the animal is resecured to the observation pedestal and the ear repositioned on the slide and returned to the Leitz microscope for evaluation of occlusion effects. The time required to complete this procedure is approximately 30 min.

Using low-power scanning, RZ and NRZ capillary loops are relocated and identified by comparing the previously recorded baseline video. Following identification, capillary loops are recorded as with baseline for the evaluation of occlusion effects. Sequential recordings are made of capillary loops in each zone and continued on an hourly basis for the duration of the occlusion period.

The occlusion/reperfusion process has been evaluated in 10 animals and in eight of these, complete flow stasis has been induced in RZ capillaries. In the two cases where complete flow stasis could not be achieved with suture ligatures alone due to collateral flow of secondary or tertiary branches, a complete stasis was effected by placement of a removable microhemoclip on the ear, positioned up to and slightly past the region of the central artery.

At the end of the occlusion period, the animal is once again placed under the surgical microscope and reperfusion is effected by the careful removal of suture ligatures. This procedure takes approximately 5–10 min. The animal is then resecured to the observation pedestal for reperfusion data acquisition. The RZ and NRZ are again relocated as previously described and video recordings are

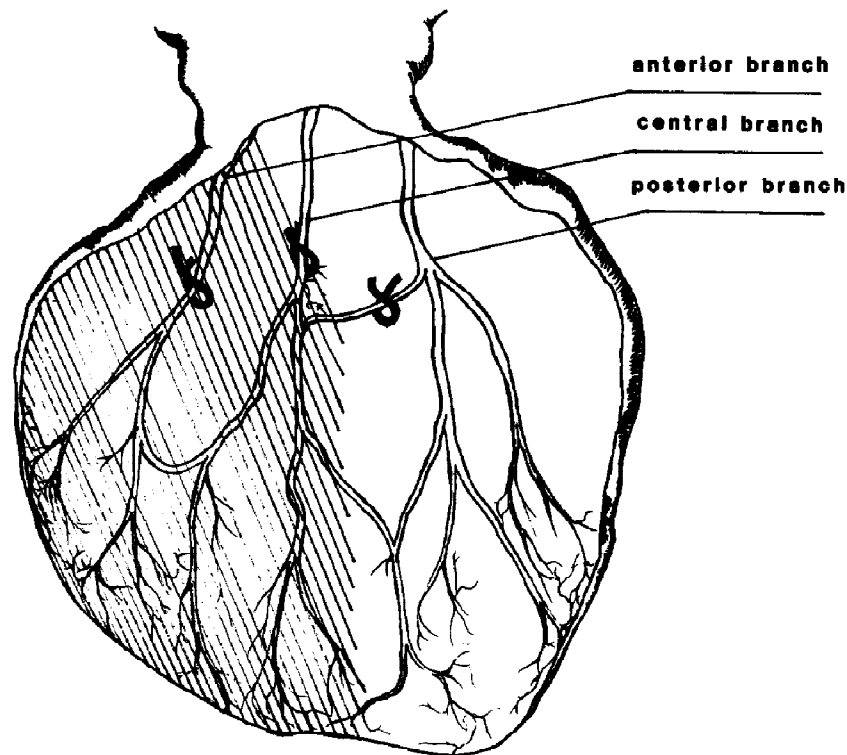


FIG. 2. Schematic diagram of the ear vasculature. Cross-hatched area represents the tissue region subject to ischemia (risk zone) following closure of the arteriolar ligatures illustrated. Capillary parameters can be determined before, during, and after ligature release in both risk and non-risk zones for comparison.

made of each zone for analysis of reperfusion. Upon completion of the study, the animal is de-instrumented and returned to the cage to recover. Follow-up recordings can be made subsequently.

## DISCUSSION

The primary purpose of this work was to develop an effective method of producing regional microvascular ischemia in the skin microvascular bed, whereby ischemic vs nonischemic tissue could be comparatively assessed and studied on an acute, as well as chronic, basis. The homozygous hairless mouse ear subserved this purpose well in that an intact tissue which required minimal intervention for observing the microcirculation could be used. This method allowed for the location and evaluation of the same capillary loops within prescribed zones (RZ and NRZ), which could be reidentified and analyzed after ischemic and nonischemic manipulations were performed. With this model a variety of capillary parameters can be used to assess ischemic effects including pre- and postischemic durations of flow in individual capillaries and capillary blood velocity and diameter. In summary, the method herein described is useful for the study of regional ischemic effects of varying duration on skin microcirculation. Its advantages include the absence of direct injury to the microvasculature under study as in compressional methods, the ability to locate and relocate well-defined microvascular regions including individual capillaries and capillary loops during

both acute and chronic studies, and direct verification of zero flow states within ischemic capillaries.

### ACKNOWLEDGMENTS

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