Reactive Hyperemia in Legs of Dogs: EFFECTS OF TEMPERATURE AND INTRAVASCULAR TENSION

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After observing that reactive hyperemia (RH) in the human forearm increased in duration and magnitude as the preceding period of arterial occlusion was prolonged, Lewis and Grant suggested that this stasis vasodilatation resulted from progressive accumulation of "normal products of tissue metabolism . . . which are removed normally as fast as they are formed." Although indirect evidence of such vasodilator metabolites leaving an ischemic vascular bed has been reported, their precise nature has not been resolved. Other workers have proposed that anoxia resulting from vascular occlusion may account for RH, either by direct action on muscle fibers of blood vessels, or in some indirect manner.

Freeman proposed that metabolism in tissues not receiving blood would incur a blood flow debt equivalent to the volume of blood which would have perfused those tissues had the occlusion not been applied. In chronically sympathectomized human hands the excess blood flow following 10-minute arterial occlusion did quantitatively repay this debt. Likewise, quantitative RH debt repayment was observed by Abramson et al. in human forearms enclosed in water baths with temperatures of 14 to 32°C. At 43°C, however, the debt was only partially repaid. Apparently at high temperatures reflex vasodilatation increased resting blood flow rates considerably above that expected solely from increased metabolism caused by heat so that the calculated blood flow debt far exceeded metabolic requirements of the tissues. This deceptively great debt of thermal vasodilatation was not repaid. Myogenic relaxation of vascular smooth muscle resulting from decreased transmural tension as intravascular pressure falls during arterial occlusion was first observed by Bayliss, and has also been reported to contribute to production of RH.

The present work was done to describe RH quantitatively as it occurs in hind legs of anesthetized dogs at several body temperatures, and to study the effects of maintaining normal intravascular tension with arterial or venous blood during circulatory stasis. It was anticipated that these approaches might resolve the relative roles of mechanical and chemical factors influencing RH.

Methods

Mature mongrel dogs of both sexes were anesthetized with 0.6 to 0.7 ml/kg allobarbital-urethane, and a tracheal cannula inserted. A carotid artery was cannulated for recording mean arterial blood pressure with a mercury manometer. The cephalic vein was cannulated to permit infusion. Preparatory to studies of blood flow the femoral arteries and veins were dissected free to their points of distal bifurcation. Small branches of femoral vessels were ligated and cut, but saphenous vessels were preserved. The profunda femoris vessels were ligated. In order to eliminate all collateral circulation to the leg, a one-half inch nylon web tourniquet was applied around the thigh, but this excluded the femoral artery and vein. Thus, all blood traveled by way of these two vessels.

Bubble flowmeters similar to those of Brunner, constructed of polyethylene tubing of 4.3 mm I.D. and a length of 1.1 m were placed in the femoral veins after administration of sodium heparin in a dosage of 5 mg/kg. An air bubble of approximately 0.1 ml was introduced into the distal end of the flowmeter with a syringe, and the time required for it to traverse a length of tubing of known volume was measured to the nearest
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0.1 second. The control rate of blood flow was considered to be 100%; experimental blood flow rates were expressed as follows:

\[
\text{flow rate (percentage of control)} = \frac{\text{control time (seconds)}}{\text{experimental time (seconds)}} \times 100
\]

Alternatively, absolute flow rates were calculated as follows:

\[
\text{flow rate (ml/min)} = \frac{\text{flowmeter volume (ml)}}{\text{time (sec)}} \times 60
\]

At the beginning of each experiment rectal temperature and blood pressure were measured. Six blood flow determinations were done at 30-second intervals and the mean taken as control rate of flow. Circulatory stasis was then produced by clamping the femoral artery or vein. At the onset of arterial occlusion a bubble was injected into the flowmeter and observed for movement which would indicate inflow to the leg through collateral channels. If flow was observed, the tourniquet was tightened until complete circulatory stasis was obtained. After 5 or 10 minutes of occlusion the vessel clamp was removed. At 10 and 30 seconds after restoration of circulation the rate of blood flow was recorded. Flows thereafter were recorded at 30-second intervals until time equal to the occlusion period had elapsed. A minimum of 15 minutes was allowed for recovery after 5-minute occlusions; 30 minutes were allowed between 10-minute occlusions. Similar procedures were followed in venous occlusions.

A group of 14 dogs was used to study RH under basal conditions. Control blood flows were recorded, following which 5- or 10-minute arterial occlusion was applied. The RH ensuing on removal of the occlusion was then observed until control levels of blood flow were obtained again.

Experimental group A included 24 dogs in which the possible role of temperature-labile factors in RH was investigated. After the animals had been anesthetized, hyperthermia was induced by exposure to cold air. When rectal temperatures reached about 28°C the animals were prepared for blood flow studies as described above. RH was then induced by 10-minute occlusion of the femoral artery. The animals were then rewarmed by heating pads and infrared radiation applied to the trunk. RH was measured at several body temperature levels from 30 to 40°C. Experimental group B included 8 animals subjected to 5- or 10-minute occlusion of the femoral vein of one leg and a simultaneous occlusion of the femoral artery of the opposite leg in order to evaluate the role of intravascular tension in RH.

Experimental group C was composed of 6 dogs in which a pulsatile finger pump was used to maintain arterial blood pressure in the leg during the occlusion period. In these preparations the femoral arteries were cannulated with T-tubes to permit perfusion. Venous blood was withdrawn through a polyethylene catheter inserted into the vena cava via the left femoral vein and stored under oil at 37°C. Normal arterial pressure and transmural tension were maintained in one leg by clamping the femoral artery and vein, and then pumping sufficient venous blood into the femoral artery below its occlusion to keep the pressure at the level of that in the carotid artery. The pump was adjusted to provide pressure pulsations equal to the heart rate. The femoral artery of the opposite leg was clamped and intravascular pressure permitted to fall to its resting level during the 5- or 10-minute occlusion period.

Experimental group D consisted of 6 animals which were prepared as those in group C but in which blood for infusion was obtained from the left femoral artery. The femoral artery and vein were clamped in one leg as in group C and stored arterial blood pumped into the femoral artery as described above. The femoral vein of the opposite leg was clamped for an equal time.

In order to quantitate RH, blood flow debt repayments were calculated according to the method of Freeman. The blood flow debt (in ml) incurred during the period of circulatory stasis was determined by multiplying the preocclusion control rate of blood flow (ml/min) by the duration of the occlusion. In presenting the results, RH blood flow was graphed as percentage of the preocclusion rate of flow against time after removal of the occlusion. The area between the resulting curve and the 100% or control flow rate was determined; this area represented the volume of blood flow in excess of control flow during the hyperemic period. The percentage blood flow debt repayment was the volume of blood in excess of the control \( \times 100 \) divided by blood flow debt. Duration of RH was taken as the time required for postocclusion blood flow rate to fall to preocclusion rate.

Results

REACTIVE HYPEREMIA IN NORMAL DOGS

A group of 14 normothermic dogs, rectal temperature 37 to 39°C, was subjected to 5- and 10-minute occlusions of the femoral arteries. The mean duration of RH which followed 5-minute occlusions was \( 4.9 \pm 0.4^* \) minutes, during which the blood flow debt was \( 49.9 \pm 4.8^* \) repaid. Following 10-minute arterial occlusions RH lasted \( 9.7 \pm 0.5 \) minutes and blood flow debt was \( 49.5 \pm 4.9^* \) repaid. A typical re-

* Values are means ± standard error of the mean.
FIGURE 1

Time course in leg blood flow after termination of 10-minute arterial occlusions in group A dogs at graded body temperatures. Lines represent mean readings from 24 animals. Numerals are rectal temperatures. Arrow indicates end of occlusion period.

response is the flow curve shown in figure 1 for 38°C.

EFFECTS OF BODY TEMPERATURE ON RH

Plotted in figure 2A are the mean durations of RH following 10-minute femoral artery occlusions at several body temperatures in group A dogs. The shortest period of RH (3 min) was seen at 32°C, and as rectal temperatures rose from 34 to 40° C, durations increased. Mean blood flow debt repayments in the same experiments are shown in figure 2B. Least repayment of the debt occurred at 32 to 34°C; repayments then increased as rectal temperatures were elevated from 34 to 40°C. The momentary means of blood flow at each temperature are shown in figure 1.

EFFECT OF TRANSMURAL PRESSURE ON REACTIVE HYPEREMIA

Mean blood flow rates of animals of groups B, C and D following 5-minute periods of circulatory stasis produced by four methods are compared in figure 3. Since flow rates following 10-minute occlusions were similar to these, except more prolonged, they are not presented. As seen in this figure, both magnitude and duration of blood flow which followed 5-minute venous occlusions were significantly less than those which followed an equivalent period of arterial occlusion. Maintaining intravascular pressure equal to carotid blood pressure by pumping venous blood into the femoral artery below the occlusion did not significantly alter the configuration of the postocclusion blood flow curve from that seen after simple arterial occlusion. However, pumping in arterial blood reduced hyperemia to the level of that following a simple venous occlusion. The percentage of blood flow debt repaid, shown in figure 4, was about the same following 5- or 10-minute occlusions of the femoral artery, or following arterial occlusions during which intravascular pressure was maintained with venous blood. The debt repayments which followed release of venous occlusions were about one-fourth of those found after arterial occlusions. Debt repayments following occlusions during which intravascular pressures were maintained by pumping arterial blood into the femoral arteries were similar to repayments following simple venous occlusions.

Mean preocclusion control rates of flow,
and the volumes of arterial or venous blood pumped during 5- and 10-minute occlusions are shown in table 1. The mean volume of blood pumped in five minutes was slightly less than one minute of control flow; that pumped during 10-minute occlusion was about 1.3 times one minute of control flow. There was no significant difference between the means of arterial and venous blood volumes that were pumped to maintain intravascular pressure. The percentage of blood flow debt repaid, as can be seen in the table, was increased approximately four times when venous blood, rather than arterial blood, was used to distend the occluded vascular bed. This was true in both the 5- and 10-minute occlusions.

**Discussion**

In normothermic dogs blood flow debts incurred by 5- and 10-minute occlusions of the femoral arteries were 49.9 and 49.5% repaid, respectively, during the following RH. These results are less than would be expected according to the view of Freeman that debt repayment is determined by the metabolic deficit of ischemic tissues, which can be estimated from the basal rate of blood flow measured before application of the occlusion. It is
Comparison of mean repayments of blood flow debt following 5- and 10-minute occlusions of the femoral artery or femoral vein. Also compared are debt repayments which resulted when intravascular pressure during occlusion was maintained by pumping in either arterial or venous blood. Brackets indicate standard error of the mean.

apparent, however, that the percentage of blood flow debt repaid will be determined by the accuracy to which the actual metabolic debt was indicated by the control blood flow volume. If the preocclusion rate of blood flow exceeded the metabolic needs of tissues, for example, a blood flow debt would be calculated which is in excess of the true needs of the tissue. Consequently, there would be only partial repayment of the calculated blood flow debt.

Siems and Kosman,12 and Ederstrom et al.13 have shown that deep anesthesia caused blood flow to the hind legs of normal dogs to increase independently of metabolic pressure of the tissues, apparently because of depression of central vasomotor control. It is likely, therefore, that in our animals anesthesia depressed nervous vascular tone and caused basal flow rates to increase to levels above actual metabolic requirements of tissues, thus fallaciously increasing the calculated blood flow debt. Since the needs of the ischemic tissues for blood remained unchanged, repayment of the metabolic debt may have been adequate, although the calculated debt was not fully repaid. Similarly, in acutely sympathectomized cat limbs where central vasomotor control was lost and local tone not yet established, it has been found that the increased resting blood flow made RH following arterial occlusion far less conspicuous than in normally innervated limbs.9

In our experiments the periods of RH following 5- and 10-minute arterial occlusions had mean durations of 4.9 and 9.7 minutes, respectively. This is in agreement with studies done by Montgomery et al.,14 who found that in normothermic, anesthetized dogs RH duration in the hind legs was 75 to 100% of the length of the preceding occlusion.

Since anesthesia depresses the thermoregulatory and vasoconstrictor response to cold, lowering the body temperature of the deeply anesthetized animal would be expected to decrease tissue metabolic activity, but without a corresponding reduction of blood flow (from which blood flow debts were calculated). This would account for the progressive decrease of blood flow debt repayment seen when the animals' core temperatures were varied from 40 to 34°C (fig. 2B). It has been reported15 that when body temperatures were lowered to 25 to 32°C, cold began to exert a direct constrictor effect on vascular smooth muscle. This intrinsic vasoconstriction may partially compensate for loss of vasomotor tone caused by anesthesia, and reduce basal blood flow rates from which blood flow debts were calculated, thus accounting for the somewhat greater debt repayment seen at 30 than at 32 or 34°C. Duration of RH was least at 32°C, and then increased nearly linearly as animals were rewarmed from 32 to 40°C. Prolongation of the hyperemic period, rather than greater postocclusion flow rates, accounted for the progressively larger debt repayments found when rectal temperatures rose (figs. 1 and 2A). The observation that RH varied with temperature suggests that thermolabile factors determined the duration of postocclusion hyperemia.
The blood flow curves in figure 3 show that RH following 5-minute occlusions of femoral veins of dogs was less intense and briefer than that produced by an equivalent arterial occlusion. Furthermore, mean blood flow debt repayments following 5- or 10-minute occlusions of femoral veins were only about one-fourth of those following femoral artery occlusions of equal duration (fig. 4). It has been postulated that the relatively small RH which follows venous occlusion occurs because vessels retain most of their tone, in accordance with Bayliss’ findings, since continuous high transmural pressure maintains the stimulus for myovascular contraction during the period of stasis.

Tension on the vascular wall has been implicated by several workers as a determining factor in RH. For example, occlusion of the inferior vena cava in cats has been found to induce RH of less intensity than did occlusion of the aorta supplying the same vasculature. Likewise, packing human forearms with arterial blood reduced the hyperemia that followed release of complete occlusions lasting up to 10 minutes. In both intact and acutely denervated dog forelimbs it was also found that packing the vessels with arterial blood decreased RH that followed occlusions of short duration.

It is possible, however, that vasodilatation occurring during venous occlusion might be reduced not only by maintenance of high intravascular tension, but also by a persistent but limited flow of arterial blood into the obstructed vascular bed as its capacitance vessels distend. In order to investigate the possible significance of such arterial inflow, venous blood, rather than arterial blood, was used to maintain intravascular tension at the level of carotid pressure by means of a pulse pump. RH following this type of circulatory stasis was not significantly different in any respect from that following simple arterial occlusion (fig. 3). Failure of high intravascular tension to reduce RH quantitatively was due to use of venous blood and not the result of (1) an artifact of the pumping procedure, (2) dilution of vasodilator material accumulated during stasis by increased blood volume of the congested leg, or (3) compression of limb vessels by edema fluid. This was shown by the reduction of RH to the magnitude seen after simple venous occlusion when arterial blood was pumped into the femoral artery of such a preparation (figs. 3 and 4).

From these results it is evident that diminution of RH following venous occlusion, as compared to that following arterial obstruction, is dependent upon inflow of arterial blood, perhaps bearing with it some component, such as oxygen, that is necessary for maintenance of vascular tone, rather than to the mechanical effect of vessel wall tension. One might anticipate that the sum of the arterial blood pumped into the femoral vascular bed during occlusion plus the hyperemia following occlusion would equal the debt repayment observed after simple arterial occlusion. (This would actually be an overestimate of the blood available to the leg, since most of the blood pumped into the leg will also be measured during RH, and thus accounted for twice.) However, when the blood pumped was added to the excess flow during RH, the sum was only about one-half the debt repayment which occurred in the same leg following simple arterial occlusion (table 1). Thus, a limited quantity of arterial blood available during occlusion seemed relatively more potent for reducing RH than similar blood available after release of the occlusion.

RH following short periods of arterial occlusion has been reported by several experimenters to result from myogenic relaxation that followed reduction of pressure in the vessels. In our experiments this effect appeared to be masked by more potent metabolic factors acting during the long occlusion period. The probability that vasodilation is of metabolic nature was substantiated by the temperature characteristics of RH, and by the finding that hyperemia differed markedly when a high intravascular pressure was maintained by venous rather than arterial blood during the period of occlusion.
Summary

1. In normal dogs 5- and 10-minute occlusions of the femoral artery were followed by periods of reactive hyperemia (RH) whose duration closely approximated the length of occlusion. The resulting vasodilatation repaid about 50% of the blood flow debt, which was calculated from the flow rate measured just prior to occlusion.

2. When the animal's body temperature was lowered to 32°C, the duration and percentage blood flow debt repaid decreased to about one-half and one-third, respectively, of that found at 40°C. Observations of RH during rewarming revealed progressive increases in debt repayment and duration of the hyperemic period. The temperature characteristics of RH suggested that thermolabile factors were responsible for the vasodilatation that resulted from arterial occlusion.

3. Venous occlusion was followed by RH that was considerably reduced, resulting in a debt repayment of about one-fourth of that attained after arterial occlusion. The possibility that high transmural pressure might be a factor in reducing the intensity of RH that followed venous blockage was investigated. However, when a pressure equivalent to that in the carotid artery was maintained in the vasculature by pumping venous blood into the leg with its artery and vein clamped, the RH following restoration of blood flow closely resembled that induced by arterial clamping only. When arterial blood was infused, RH was reduced to the level seen after simple venous occlusion. It was concluded from these results that during venous occlusion some arterial blood entered the vascular bed, and brought with it chemical factors that reduced the degree of vasodilatation. Since total absence of arterial blood inflow was required to cause maximal dilatation, it may be that oxygen lack was involved in relaxation of the vascular wall, as has been implied by the work of investigators using other techniques.

References

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