Site of increased vascular resistance during isometric muscle contraction

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GRAY, SARAH D., ERIK CARLSSON, AND NORMAN C. STAUB. Site of increased vascular resistance during isometric muscle contraction. Am. J. Physiol. 213(3): 683-689. 1967.—We studied the intramuscular vessels during maximal tetanic contraction to localize the site of increased flow resistance. We examined the microcirculation of rats whose calf muscles were rapidly frozen before, during, or after maximal contraction. We found no evidence of compression of the microvasculature. Indeed, counts of functional capillaries per muscle fiber showed significantly more open capillaries within 5 sec of the onset of a single maximal tetany compared to the resting condition. In anesthetized dogs we made arteriograms and venograms before, during, and after single maximal tetanic contractions of the calf muscles. We found numerous localized compressions and pinching of both arteries and veins as they entered the muscle or passed between muscle fasciculi. The defects were reproducible and were never seen when the muscle was relaxed. The evidence supports the view that the vascular resistance increase seen during strong muscle contraction is chiefly in the larger supplying vessels rather than in the microcirculation.

angiography; arteries; blood circulation; blood vessels; capillaries; microcirculation; muscle blood flow; tissue oxygen supply; veins

In the previous paper we confirmed that isometric contraction of skeletal muscle causes an increase in resistance to blood flow through that tissue especially when contraction is maximal. The site of increased vascular resistance has been thought at various times to be in the large veins, large arteries, the whole microvascular bed, or the capillaries. Gaskell (7) postulated that it occurs in the venous system and most others who have measured flow directly attribute the spurt in venous outflow at the onset of contraction to a compression of the large veins. Gaskell also made microscopic observations of the surface vessels of the mylohyoid muscle in the frog. During contraction he found small arteries dilated and the venous flow arrested. Heileman (10), using the same technique, found that only venules parallel to muscle fibers were compressed. Anrep et al. (2) and Drury et al. (5) examined intestinal smooth muscle and found that local, segmental contractions emptied the capillaries in the muscularis mucosa and in the main layers of intestinal smooth muscle. Krogh (15) noted the presence of valves even in small veins and concluded that muscle contraction causes blood to move from the capillaries and veins toward the heart and relaxation permits the emptied capillaries to refill. Pearson (20) also suggested that capillaries are emptied during contraction. Barcroft (3) suggested that both large arteries and veins were "nipped" and Start and Holmes (27) considered that the whole microvascular bed was compressed.

A few workers have used angiography to determine the width and configuration of the vascular bed during muscle contraction. Frey (6) compared arteriograms of the relaxed and contracted hindlimbs of the dog and found that the contracted limb showed generalized vasodilation, including the smallest branches of the arterial tree. Mazella (18), on the other hand, made angiorams of human calf muscles during contraction and found that arterial flow decreased and that there was no dilation of the terminal vascular bed. Almén and Nylander (1) demonstrated compression of veins in human leg muscles.

The purpose of our investigation was to examine the "living" intramuscular vessels during maximal tetanic contraction in order to localize the site of increased flow resistance. We studied the entire vascular bed of the lower leg muscle in rapidly frozen calf muscles of anesthetized rats before, during, and after maximal
contraction. The rapid freezing enabled us to immobilize the vascular bed under known conditions of muscle tension. We were interested mainly in quantifying the effects of sudden strong contraction on the capillary bed. We studied the larger arteries and veins of the lower leg muscle in anesthetized dogs by rapid serial angiography before, during, and after maximal contraction.

**METHODS**

**Rats.** We anesthetized young male Wistar rats (wt 70–100 g) with an intraperitoneal injection of 6 mg sodium pentobarbital (Pentosol)/100 g body wt, removed the skin from the left thigh and calf, and ligated and cut the sciatic nerve in the midthigh region. We cut the tendinous insertion of the biceps femoris muscle on the tibia, reflected it to expose the gastrocnemius muscle and ligated the branch blood vessels supplying the distal end of the biceps. We mobilized and cut the Achilles tendon and tied it with a heavy silk thread, covered the leg with cotton moistened with warm saline, and transferred the animal to an apparatus that rigidly held the leg flexed at right angles at the knee and ankle by bone clamps. The rat was in the prone position so that the posterior surface of the calf muscles faced upward. We tied the ligature from the Achilles tendon to a force strain gauge (Grass Instrument Co.) with a slight resting tension (about 10 g) on the calf muscles. We set up a continuous slow drip of 0.9% NaCl solution at 37°C over the exposed muscle. We attached bipolar shielded silver electrodes to the distal stump of the sciatic nerve so that we could stimulate the leg muscles via a square-wave stimulator (Grass model S-4). Supramaximal stimulation (4–5 V, 0.2–0.4 msec duration) gave a maximum tetanus when the pulse frequency was 30–40 cycles/sec.

When we were ready to freeze the leg we discontinued the saline drip and positioned a funnel without a spout over the leg with the open tip directly over the posterior fleshy part of the gastrocnemius. We poured liquid propane, cooled to −180°C in liquid nitrogen, continuously through the funnel over the calf and collected the run-off in a beaker under the leg (28).

We tested six rats to determine the freezing rate of the calf muscles by inserting three silver-covered, copper-constantan microthermocouples (Bendix Corp.) into the belly of the calf muscles to depths of 1, 3, and 6 mm from the posterior surface. We determined the time required for each thermocouple to reach a temperature of −10°C. We also followed the record of muscle tension to see whether freezing affected the tension.

There were three experimental groups. In group 1 we froze the muscles in the relaxed state without nerve stimulation; in group 2 we froze the muscles during postcontraction hyperemia induced by intermittent stimulation of the nerve at the rate of 2/sec for 2 min; in group 3 we froze the muscles within 5 sec of the onset of a single maximal tetanic contraction.

When the leg was completely frozen, we separated the calf muscles in a cryostat at −30°C and either freeze-dried them at −30°C (Canalco Industrial Corp.) or fixed them by freeze-substitution in nonaqueous solvents at −40°C (12).

We made thick sections of the frozen-dried muscles by clearing them in xylol and hand sectioning them longitudinally at 1-mm intervals with a razor blade. In the sections mounted in a well of Permount on a microscope slide we could see the entire vascular supply down to the arterioles and venules using low-power stereomicroscopy.

From the freeze-substituted, fixed muscles, embedded in nitrocellulose or paraplast we made 10-μ thick sections, both longitudinally and transversely. By staining red cells selectively with Chromotrope 2R (4) or benzidine (21) and lightly counterstaining with 0.5% fast green we could determine the functional state of the capillary bed. We used the longitudinal sections for qualitative study and the transverse sections for quantitative red cell-containing capillary to fiber (C/F) counts. We made counts in 20 different muscle fasciculi in each leg. Although we examined the entire thickness of muscle, counts are confined to the outer 2.5 mm of...
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the gastrocnemius as measured from the posterior surface since this portion froze most rapidly.

Dogs. In 13 anesthetized dogs we prepared the right leg for angiographic study in the manner described in the previous paper (6) with the following modifications: the skin of the leg was removed, the leg was wrapped in gauze kept wet with warm saline; no flowmeters were used. A Sanchez-Perez film changer was positioned directly under the lower leg. Three to four angiographies were performed in each animal with an interval between the contrast medium injections of 30 min. For each angiographic run, 5-40 ml of contrast medium were injected at a rate of 5 ml/sec. The injection rate was slow enough to permit coverage of one phase of relaxation (3 sec) and one phase of muscular contraction (3 sec). The exposure rate was 2/sec. Renographin 76% was used in the majority of the experiments, but Thorotrast and Micropaque were used in one examination each in order to rule out any gross effect of the Renographin as such. In eight dogs the arteries alone were examined; in five both arteries and veins were demonstrated. The measurements of vascular calibers were corrected for geometric magnification; the average magnification factor was 10%.

RESULTS

Rats. The rat calf muscles, measuring 6–6.5 mm thick from posterior surface to tibia, froze solid in approximately 7 sec. However, the outer 2.5 mm froze in about 1 sec. Since the main vascular supply to the calf is on the surface it froze at the same time as the muscle surface. Tension records is either relaxed or contracted conditions did not show any significant changes during freezing.

Examination of the thick sections showed that in the relaxed state before or after contraction the large arteries and veins curve smoothly over the calf surface and along the cleavage plane between the two heads of the gastrocnemius. We found major kinks or compressions in these vessels and their principal tributaries in 13 of 18 contracted muscles (Fig. 1) compared to 1 of 18 in each of the relaxed groups (groups I, 2). The very small arteries, arterioles, venules, and veins did not appear to be affected by contraction.

In the thin longitudinal sections there are important qualitative differences in appearance among the three experimental groups. In group 1 (relaxed) the capillaries are mainly long straight tubes coursing parallel to the muscle fibers with numerous anastomoses around the fibers. The erythrocytes are separated along the length of the capillary. The cells have elongated shapes suggesting that the capillaries were narrower than the red cells (Fig. 2A). In group 2 (postcontraction hyperemia) the capillaries are also long straight tubes but there are more of them in evidence with more anastomoses with one another. The erythrocytes are closer together and the cells are not as elongated as in group 1 (suggesting increased capillary diameter) (Fig. 2B). The major difference between group 3 (contracted muscle) capillaries and those of the other two is the change from long straight tubes to sinuous ones. In most instances they appear as a simple wave, but in a few they are actually coiled. The cells are closer together than in group 1 and as close as group 2. The cells are not elongated, suggesting the capillaries are as wide or wider than in the relaxed condition (Fig. 2C).

The C/F counts in thin transverse sections gave quantitative confirmation of the qualitative picture. The average values and standard error for 20 animals in each group are listed in Table 1.

Dogs. Figures 3, 4, and 5 show angiograms of relaxed and contracted leg muscles (the calf and anterior tibial groups). During contraction the calf muscle shortened an average of 4 % as measured from reference marks on the surface of the muscle. The arteries sup-

FIG. 2. Longitudinal thin sections of rat gastrocnemius muscle under three conditions. A: group 1 (relaxed); B: group 2 (postcontraction hyperemia); C: group 3 (isometric tetanus). All sections have same magnification (approx. X 400).
TABLE 1. Red cell-containing capillary/fiber ratios in rapidly frozen rat gastrocnemius muscle under three conditions

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Expts</th>
<th>Capillary/Fiber, Avg ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Relaxed</td>
<td>20</td>
<td>0.90 ± 0.06</td>
</tr>
<tr>
<td>2. Postcontraction hyperemia</td>
<td>20</td>
<td>1.87 ± 0.07</td>
</tr>
<tr>
<td>3. Isometric tetanus</td>
<td>20</td>
<td>1.26 ± 0.09</td>
</tr>
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Plying the calf and anterior tibial muscles showed short segments of marked compression (nipping) as well as longer segments of narrowing. The nipping occurred at points where the vessel entered a muscle or a group of muscles. The veins usually showed defects at the same level as the accompanying artery. Both arterial and venous defects were reproducible each time the experiment was repeated in the same animal. The compressions were seen on the first film exposed after the onset of muscular contraction, that is, within 0.5 sec. When we induced contraction before the injection of contrast media only the arteries exterior to the contracting muscles filled.

The vessels most affected by the contraction were the penetrating branches of the caudal femoral artery down to 1 mm diameter and the lateral saphenous vein down to 1.5 mm diameter. The main cranial tibial artery was sometimes nipped as it passed between the calf muscles and the tibia.

DISCUSSION

Rapid freezing of living tissue at some known point during its activity cycle is a useful histologic procedure for studying the interdependence of structure and function (19, 28). It is important to show in each application that the freezing is rapid enough to preserve the event under study. In our investigation important events to be preserved are skeletal muscle tension and vascular configuration. Although the whole rat calf required about 7 sec to freeze solid, the outer 2.5 mm froze in 1 sec, as determined by our microthermocouple studies. This outer shell forms a rigid casing and together with the simultaneous freezing of the supplying vessels prevents changes in muscle configuration and movement of blood in or out of the leg. The fact that measured tension in either the relaxed or contracted state is not affected by the freezing is additional evidence that muscle configuration has been maintained. Finally, since we are comparing similar groups of muscles and since freezing is the same in all groups the differences noted can be compared relative to each other even if absolute values are indefinite. As far as vascular tone is concerned the available evidence suggests this is much less likely to be affected by freezing than is skeletal muscle. Keatinge (13) studied fast cooling of isolated arteries and showed that a general relaxation occurred over a 30-sec period.

Kulka (16) compared the vessels in living rabbit ear before and after rapid freezing and did not find any significant alteration. Ice crystal growth with subsequent cytologic distortion is minimal. No intravascular ice has been seen by us and even the ice crystals within the skeletal muscle cells are small and the cells do not show any noticeable difference from control rat calf muscles fixed without freezing.

A major criticism of older histologic methods of clamping the blood supply of an organ and fixing it at room temperature is that the event of interest is not preserved. In contrast, rapid freezing preserves the event and permits a comparison of the relaxed and contracted state of a muscle.

FIG. 3. Arteriogram of leg muscles in a dog showing relaxed (R) and contracted (C) states. Arrows point to arterial segments which are nipped or generally narrow during muscular contraction. Twenty milliliters Renographin 76% were injected into the femoral artery at a rate of 5 ml/sec.
temperature is that tissue and vascular pressure relations are lost and the slowness of fixation may lead to reapportionment of the blood volume within the vasculature especially if the fixatives cause differential changes in compliance or contracture. The rapid freezing technique overcomes these difficulties.

On the other hand, the presence of red cells in a capillary is not absolute proof that the capillary is open to flow, that is, there might be stationary red cells in capillaries with closed precapillary sphincters. In a series of preliminary experiments we looked for this possibility by comparing C/F counts in comparable resting control muscles by classical India ink injection and by red cell counts. We anticipated the maximum number of stagnant red cell-containing capillaries in this situation and thus should obtain higher C/F counts from the red cell data. Actually the data showed slightly higher C/F using ink. We concluded that the presence of red cells in closed capillaries was not a significant problem within the limits of our experiments.

Our qualitative descriptions of the capillary bed under differing conditions confirm a variety of reports by previous investigators. For example, Krogh (15) stated that in rhythmically contracting muscle the capillaries have a wider diameter and permit the red cells to pass through undeformed. Our pictures suggest wider capillaries and less red cell distortion in both the tetanically contracted and hyperemic states. Since we did not stain specifically for capillary outlines we do not have quantitative measurements of capillary diameter. We interpret our qualitative findings as indicating no net increased compressive forces on the capillaries during contraction.

In addition to the distortion of red cells passing through capillaries of smaller diameter than the cells, as in bolus flow (23), they may also be deformed into hollow paraboloids by the plasma velocity profile as Guest and his co-workers have demonstrated in relatively large mesenteric capillaries (9). We cannot absolutely differentiate between these two effects in our studies since both are occurring simultaneously. In the contracted state flow velocity is reduced almost to zero as indicated by the failure of our contrast media to enter the contracted dog muscle and by the results of our previous paper (8) in which maximal contraction markedly decreased total muscle inflow and outflow. But the individual capillaries appear to be wider. In postcontraction hyperemia capillary plasma velocity is probably not much altered because, although total flow is markedly increased (2- to 3-fold) the capillary cross-sectional area is also increased about threefold as judged from the increased diameters and the doubling of C/F count ratios.

Spalteholz (26) and Krogh (14) reported that isotonically contracting muscle had tortuous capillaries as compared to the straight capillaries of resting muscle. We have also noted bending and coiling of capillaries in isometrically contracted muscle even though the over-all contraction is nearly isometric (4 % shortening as measured in the dog calf). Hill (11) pointed out, however, that even in isometric contraction the contractile elements shorten and the series-elastic elements lengthen. The capillaries are in parallel to the contractile element and may be regarded as a sensitive indicator of the contractile element shortening.

The new quantitative data on C/F ratios during maxi-
normal isometric tetanus is crucial. Previous workers using injection methods have examined C/F in resting and postcontraction hyperemia but, of course, have been unable to do so during contraction. Martin, Woolley, and Miller (17) found C/F at rest in dogs to be 0.64 and in postcontraction hyperemia to be 1.23. The maximum C/F after dilating all the precapillary sphincters with amyl nitrite was 2.0. Schmidt-Nielsen and Pennycuik (25) found that in dead rat muscle the maximal ratio was 1.2 for white muscle and 2.5 for red muscle. Recent data obtained by Renkin and Rosell (23) for estimating the extent of the functional muscle capillary bed at rest and during postcontraction hyperemia using $^{85}$Rb extraction show approximately a twofold increase between these two conditions.

The rapid-freeze method should be a very accurate means of determining the functional capillary bed because it avoids all of the problems of foreign injectates, uses the animal's own pulsatile perfusion pressure, and stops activity very quickly. However, in transverse section only capillaries containing erythrocytes are counted and if a capillary has a long column of plasma between cells it is possible to underestimate the actual number of red cell-containing capillaries. In our experience this difficulty would occur only during control muscular relaxation (Fig. 2A) and even then would be minimal because the intererythrocyte distance in a single capillary is seldom greater than 10 μ (the section thickness).

Our data for resting muscle (C/F = 0.93) and for muscle during postcontraction hyperemia (C/F = 1.87) are well within the range reported by others noted above. It is therefore likely that the results during maximal tetanus (C/F = 1.26) are reliable.

We conclude that strong isometric contraction does not squeeze, kink, or otherwise cause blood to leave the capillary bed.

The accumulated evidence from both the thick rat muscle sections and the angiography in the dog provide a clear, logical, and seemingly indisputable basis for the rise in vascular resistance during muscle contraction. Most of the defects are seen in large vessels of the type that pass between muscle groups and fasciculi. We believe that changes in the orientation of these structures cause the vessels to twist, kink or be locally compressed. The reproducibility of the defects in successive experiments on the same animal and the fact that both low-pressure veins and high-pressure arteries are nipped at the same point indicate that local shearing forces rather than generalized tissue pressure is involved. It also rules out localized active vascular constrictions although our previous study (8) had already demonstrated that the effects were independent of the state of the vascular smooth muscle.

One remote possibility is that the contrast medium may have induced local spasm (29); however, such spasms were never seen in the films of relaxed muscles. In series where muscular contraction was interposed between two films in the relaxed state the defects were seen only in contraction; the relaxation films were identical.

That strong muscle contraction can partially or totally occlude major arteries has been suggested before. Rohter and Hyman (24) showed that in the hand the digital artery pulse was obliterated at 25% $T_{\text{max}}$ and the radial
artery pulse at 45% $T_{\text{max}}$. They believed the mechanism was compression of the vessels between taut fascia, tendons, and bone.

There are still two questions raised by our previous study (8). Why does vascular resistance increase gradually instead of suddenly with increasing strength of muscle contraction and why doesn't equivalent passive tension nip vessels? Our surmise is that individual vessels may be occluded in all-or-none fashion, but various vessels are occluded at various tensions so the net effect is a gradual increase in vascular resistance for the muscle.

**REFERENCES**


