Local Regulation of Effective Blood Flow in Muscle

By Chester Hymon, Ph.D., R. L. Paldino, Ph.D., and Emery Zimmermann, B.A.

Blood flow through skeletal muscle is augmented characteristically during and for some time after exercise. Information on the basic mechanism of this functional hyperemia is still far from complete. It has been suggested that vasomotor nerves or local chemical imbalances are concerned in the development and maintenance of vasodilatation. Similar uncertainty exists concerning the mechanism of reactive hyperemia, the gross increase of measured blood flow immediately following an imposed deprivation of blood supply. In the case of skeletal muscle at least three alternative hypotheses have been offered to explain relaxation of smooth muscle in the local blood vessels: (a) effects of nerve impulses which originate centrally or locally, (b) direct response of smooth muscle in the resistance vessels themselves to lessened distention, i.e., the "myogenic response," and (c) local accumulation of metabolites, or depletion of nutrients or of oxygen during the period of ischemia.

Each of these theories has been questioned on the basis of experiment. For example, total chronic denervation does not significantly alter reactive hyperemia. That something in addition to the myogenic response must contribute to the total effect is suggested by evidence that a qualitatively similar hyperemia may be obtained after venous occlusion, when the distending force is augmented rather than diminished, during the occlusion. In its simplest form, any hypothesis based on local chemical alteration during ischemia implies an exact quantitative relationship between the "blood flow debt" and the "repayment during hyperemia." However, in a recent study Patterson and Whelan found repayment of the total blood flow debt ranged from 50% to 200%. This high variability could be explained if during the hyperemia some fraction of total blood flow passes through shunt vessels and so cannot contribute to restoring normal chemical balance. As a corollary of a dual circulation hypothesis one may assume that the composition of tissue fluid is maintained constant by a steady-state relation between the production and utilization of metabolites and their removal or delivery by effective blood flow. Therefore, tissue clearance measurements of increases in effective blood flow should show exact compensation for prior deficiency. Further, if increased effective blood flow could be forced, while the production of the significant metabolite is normal, the dilator should be depleted, and a subnormal effective blood flow should result when the stimulus is removed. The experiments here reported are concerned with the changes in tissue clearance before, during, and after periods of ischemia and periods of forced hyperemia.

Methods

Two techniques were used: (a) micro-tissue clearances from rat muscle, and (b) conventional clearances from muscles in rabbits.

EFFECTIVE BLOOD FLOW IN RAT SKELETAL MUSCLE

Effective blood flow was estimated by the micro-tissue clearance technique in the exteriorized spinotrapezius muscle of rats as described previously. In "reactive hyperemia" clearance was measured by following disappearance of 4% Water Blue from a single micro-injection site before, during, and for 5 to 10 minutes after a period of occlusion. Blood flow to the muscle was stopped for periods of 30 seconds to 8 minutes by increasing the tension of a polyethylene loop around the viewing cylinder. A thermostatically regulated drip kept temperature constant at 38°C.
EFFECTIVE BLOOD FLOW IN MUSCLE

"Reactive ischemia" was studied in another series of experiments in which a micro-injection of dye was made while the temperature of the drip was 38°C. After three minutes, the temperature of the drip (and muscle) was raised to, and maintained at, 41-43°C for about four minutes, then abruptly returned to 38°C. In preliminary experiments a thermistor placed beneath the muscle showed that the tissue reached drip temperature within 30 to 45 seconds.

EFFECTIVE BLOOD FLOW IN RABBIT SKELETAL MUSCLE

Reactive hyperemia was estimated by gross clearance measurements in muscle of rabbits anesthetized by intravenous administration of 50 mg chloralose and 500 mg urethane per kg body weight. Approximately 0.2 μCi in 0.2 ml NaCl was injected into the belly of the gastrocnemius muscle and the counting rate was measured by a scintillation detector placed against the skin of the lower leg. The recorded counting rates were corrected for background and the logarithms of the resulting values were plotted against time. Clearance was followed for three to five minutes to establish a control rate, then while digital pressure was exerted on the abdominal aorta or on the femoral artery for one to five minutes, and finally, while blood flow was re-established.

In a second series of experiments the blood flow through the gastrocnemius muscle was forced to high levels by acute elevation of local muscle temperature. After a control clearance had been established, an ultrasonic generator* was applied directly over the injection site. The temperature of the muscle, as measured by a thermistor needle, showed a prompt and significant increase to a constant level which was maintained for about two minutes. The ultrasonic generator was then turned off and muscle temperature returned rapidly to its original level. Clearance rate was followed continuously throughout the experiment.

In every case the clearance plot was inspected to determine if the portion of the curve after the period of ischemia, or after the period of forced hyperemia, returned to the projection of the pre-occlusion or pre-hyperemic phase of the curve.

Results

Clearance curves from typical experiments

*An ultrasound generator with a 10 cm² head was coupled to the shaved skin over the isotope injection site by an appropriate oil. The generator was set to yield 0.5 watts/cm² and rate of oscillation was 1 Mcy/sec. This instrument was made available on loan through the generosity of Dr. Rodríguez, Department of Physical Medicine, Los Angeles County General Hospital.

The data obtained in all experiments on the rat spinotrapezius preparation are listed in table 1. Data from experiments on rabbit muscle are given in table 2. Part A of each table lists the control clearance values before occlusion (K₁) and final clearance (K₂) measured two to three minutes after occlusion was released. Debt repayment, shown in the 4th column, was calculated from the individual clearance curves (figs. 1A and 2A).

It will be noted that reactive hyperemia in 7 of 11 experiments in rats showed 100% repayment, and that 15 of 18 experiments in rabbits also showed perfect responses. Duration of ischemia ranged from 0.5 to 8.0 minutes in rats, and from one to five minutes in rabbits.

Data relative to reactive ischemia are given in tables 1B and 2B for rats and rabbits respectively. Here K₁ refers to the initial control clearance rate, while K₂ represents the final stabilized clearance after temperature of the muscle had returned to normal. The extent of the compensation was calculated as in the case of the ischemia experiments, except that the constructed line was drawn through the lowest point in the forced hyperemia curve (figs. 1B and 2B). It will be noted that perfect compensation was found in 11 of 14 experiments in rats and in 10 of 12 experiments in rabbits.

Discussion

All the data reported above support the proposition that the level of effective blood flow in skeletal muscle is in a steady-state relation to some local factor which acts directly...
TABLE 1
Micro-clearance in Rats

<table>
<thead>
<tr>
<th></th>
<th>K1 min⁻¹</th>
<th>K2 min⁻¹</th>
<th>Occlusion time min</th>
<th>Repayment per cent</th>
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<tbody>
<tr>
<td>A. Reactive hyperemia response:</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>0.117</td>
<td>0.126</td>
<td>0.5</td>
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<tr>
<td>0.145</td>
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<td>0.5</td>
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<td>0.040</td>
<td>0.064</td>
<td>1.0</td>
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<td>1.0</td>
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<td>0.067</td>
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<td>0.025</td>
<td>2.0</td>
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<td>100</td>
</tr>
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<td></td>
<td>100</td>
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<tr>
<td>0.064</td>
<td>0.084</td>
<td>5.0</td>
<td></td>
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</tr>
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<td>0.074</td>
<td>0.074</td>
<td>6.0</td>
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</tr>
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<td>0.041</td>
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<td>0.043</td>
<td>0.043</td>
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<td>0.058</td>
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<td></td>
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The clearance of micro-injected Water Blue from the exposed spinotrapezius muscle of anesthetized rats before, during, and after modification of clearance. The ordinate represents the log of optical density of the layer of muscle after correction for normal light absorption. A) Records before, during (between arrows), and after the occlusion of blood flow as described in the text. Note that the final portion of the curve is coincident with the early portion of the curve. B) Records before, during, and after a thermally induced increase in clearance from the muscle. At the first arrow the temperature of the drip was increased from 38°C to 41°C. At the second arrow the temperature was acutely restored to 38°C.

The concept of a local factor whose concentration both governs, and is determined by, local blood flow.

The possibility that a local metabolite might play a significant role in the hyperemic response, following deprivation of blood flow, has been repeatedly tested in many different preparations. Lewis and Grant attributed reactive hyperemia in the skin to such a metabolite and di Palma et al. showed that the response could be strongly modified by some blood-borne factor after the release of occlusion of remote peripheral tissue. Freeburg and Hyman showed that a circulating vasodilator substance from ischemic and exercising tissue could increase the normal blood flow through the forearm in man. The studies of Wood et
TABLE 2
Macro-clearance in Rabbits

<table>
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<tr>
<th>A. Reactive hyperemia response:</th>
<th>Occlusion time</th>
<th>Repayment</th>
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<td>$K_1$ min$^{-1}$</td>
<td>$K_2$ min$^{-1}$</td>
<td>per cent</td>
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<td>0.062</td>
<td>0.062</td>
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<td>0.080</td>
<td>0.080</td>
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<tr>
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<tr>
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<tr>
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<td>0.106</td>
<td>1.0</td>
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<td>0.138</td>
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<tr>
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<td>0.084</td>
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<tr>
<td>0.210</td>
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<table>
<thead>
<tr>
<th>B. Reactive ischemia response:</th>
<th>Compensation per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_1$ min$^{-1}$</td>
<td>$K_2$ min$^{-1}$</td>
</tr>
<tr>
<td>0.029</td>
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<tr>
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<tr>
<td>0.111</td>
<td>0.111</td>
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</table>

al.\textsuperscript{17} relate the dilatation found in exercise with that found after ischemia. The hyperemia following long periods of arterial occlusion was strongly augmented by brief periods of exercise during the ischemia. Since the exercise itself was not sufficient to produce any notable local reactions or alterations in pulse or blood pressure, and since the intra-vascular pressure was already low as a result of arterial occlusion, the exercise apparently contributed to the more rapid elaboration of a vasodilator substance. These authors suggest that the same material might be produced at all times during rest and might thus account for the steady state between blood flow and metabolism.

One objection to the hypothesis of a locally produced dilator metabolite is the high variability between blood flow repayment and blood flow deficit. If the data from figure 8 of Pat-
HYMAN, PALDINO, ZIMMERMANN

180

terson and Whelan are replotted to show the
per cent debt repayment as a function of the
pre-occlusion blood flow rate, over-payment
(over 100%) usually occurs in cases of low
initial blood flow.* Such a relationship would
be expected if the initial flow were restricted
to the "nutritive bed" and if the dilator sub-
stances, produced during ischemia, opened
both shunts and nutritive vessels. Measure-
ments of total blood flow can not distinguish
between the two circulations, but our clear-
ance estimates of effective flow support this
argument. The return of the post-ischemic
curve to the projection of the control line is
strong evidence for a steady-state relationship
between the level of effective blood flow and
the local rate of accumulation of dilator.

Another argument against the concept of
simple debt repayment has been raised by the
work of Blair, Glover and Roddie.\textsuperscript{18} During
the post-occlusion period they restricted flow
through the brachial artery to limit perfusion of
the forearm to the original, or control,
level. When this partial compression of the
artery was removed after several minutes, no
hyperemia could be demonstrated. The dis-
appearance of excess metabolites in the ab-
sence of measurable hyperemia was taken as
evidence against the role of a local metabolite
in adjusting local blood flow. However, the
constant normal total blood flow which they
imposed during the early post-occlusion pe-
riod might have masked an increased effective
flow caused by diversion from the shunt cir-
culation. This question can be resolved by
repeating their experiments using clearance
measurements.

Hyman and Paldino\textsuperscript{13} showed that a lo-
cal increase in muscle temperature increases
effective blood flow greatly in excess of the cal-
culated change in local metabolism and sug-
gested a direct temperature-induced relaxa-
tion of the smooth muscle regulating effective
flow. If such an induced hyperemia in the
nutritive circulation depletes the amount of
dilator in tissue to a subnormal level, sudden

\textsuperscript{*}Mr. Donald Burnap, a California Heart Student
Research Associate, recalculated these data for us.

return to control temperature should result
in a subnormal local effective blood flow.
Further, if quantitative relationships held,
the rate of effective blood flow would be re-
stored to its control value only when the local
supply of dilator had re-accumulated. The
findings here reported support this hypothesis
with respect to both (a) the subnormal clear-
ance after the period of heating and (b) re-
turn to the original line.

In a recent study Paldino, Hyman, and
Lenthall\textsuperscript{19} showed that bradykinin increases
effective blood flow in skeletal muscle. Two
of their figures give evidence for a reactive
ischemia on withdrawal of the dilator, sug-
gest that hyperemia induced by this drug
depletes the local dilator. Preliminary stud-
ies with other dilators, including several of
the adenosine compounds, all give evidence
of "reactive ischemia." It seems possible that
infusion of the normally produced physiolog-
ical vasodilator would augment the clearance
rate, but would not deplete the endogenous
dilator because its concentration gradient be-
tween capillary blood and tissue fluid would
remain unchanged. There should then be no
"reactive ischemia" subsequent to hyperemia
induced by the infusion of the natural dilat-
ing agent.

Summary

The techniques of macro- and micro-tissue
clearance were used to study reactive hyper-
emia. Clearance of radioactive iodide from
gross injection sites in muscles of rabbits and
clearance of micro-injected diffusible dye from
the exposed, directly observed spinotrapezius
of rats gave evidence for an exact quantita-
tive repayment of effective blood flow debt.
In both types of preparation a subnormal
clearance followed a thermally induced hyper-
emia as an apparent compensation. The
exact quantitative repayment and compensa-
tion support the hypothesis that local effective
blood flow is in a steady-state relation to the
local concentration in tissue fluid of some
agent with vasodilating properties.

It is suggested that an extension of this
method to studies of vasodilatation induced

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by drugs or other chemicals might offer an approach to identification of the normal vasodilator, and to physiological proof of its exact nature.

References
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CHESTER HYMAN, R. L. PALDINO and EMERY ZIMMERMANN

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