Local Temperature Regulation of Microtissue Clearance from Rat Skeletal Muscle

By Chester Hyman, Ph.D., and Rita L. Paldino, Ph.D.

The tissue-clearance technique as a measure of effective blood flow through a tissue is obviously indirect, and the interpretation of results is necessarily based on the validity of several assumptions. One assumption is that injection into a "muscle" labels the fluid in a single uniform tissue, i.e., no connective tissue is involved. A second assumption is that clearance rate is relatively independent of the injection volume, and that the mechanics of injection do not measurably alter the circulatory features of the tissue immediately about the injection site. Warner et al. suggest that there is an inverse relationship between the size of the bleb and the clearance rate. A final assumption is that the tissue clearance is a valid, although indirect, measure of effective blood flow.

A previous report from this laboratory validated some of these assumptions by a method extending the tissue-clearance technique to microinjection sites. The highly diffusible dye, water blue, was microinjected directly into the exposed mesentery of the frog, and the rate of disappearance was followed by means of a suitable microspectrophotometer. There was no directly observable disturbance of the capillaries in the area where the injection was made. Visual estimates of the blood flow correlated with measured clearance rates under control conditions and when the circulation was altered by topical applications of histamine or epinephrine. However, the absolute clearance in the frog mesentery (0.55 min⁻¹) was considerably higher than that reported from gross injection of Na₂¹ and I¹³¹ in mammalian skeletal muscle (0.05 to 0.06 min⁻¹). This discrepancy might be an artifact due to the smaller injection volumes used, or it might reflect real differences in nutritional blood flow in these tissues, or a combination of both. At present there are no comparable data available from gross injection sites in mesentery.

In the present study, the microtissue-clearance technique, previously described, has been extended to the skeletal muscle of the rat. This makes possible direct comparison of clearance from microinjection sites with that from gross injections in the same tissue.

Since temperature is one of the factors which can significantly alter the circulation of the exposed muscle, we used this means to modify blood flow in an extension of the study of microclearance rates. The data obtained could then be compared with the established information on the influence of temperature on total blood flow through muscle.

Methods

The exteriorized rat spinotrapezius muscle preparation, which has been used for microcirculatory studies by Zweifach and Metz, was modified for the present application in several details. Rats, weighing from 90 to 100 Gm., were lightly anesthetized by an intramuscular injection of 3.5 to 4.0 mg. of sodium pentobarbital. Proper anesthetic level was maintained by occasional supplementary intramuscular doses of 2.0 mg. The skin over the thoracic vertebral was incised and carefully reflected from the layer of connective tissue overlying the spinotrapezius muscle on the right side to expose the lateral border. This edge of the muscle was teased from underlying tissues, and two cotton ligatures were placed 1.0 cm. apart, involving as little of the muscle margin as possible. The separation of the muscle from the tissue below was then completed, leaving its origin and insertion, as well as its blood supply and innervation...

*Mr. Bradley Walsh, a graduate student in this department, undertook and carried through a similar series of experiments in 1955. His data correspond to those obtained in the present study.

†One of us, Rita L. Paldino, spent several weeks in the laboratories of Dr. Benjamin W. Zweifach, Department of Pathology, New York University School of Medicine, New York, New York. We gratefully acknowledge his courtesy.
FIGURE 1
Photograph of apparatus for maintaining exteriorized spinotrapezius muscle of the rat. Shows anesthetized rat in position on board with muscle draped over viewing chamber. Also note drop of irrigating fluid on thermistor and arrangements for securing ligatures. The rectangular opening permits positioning of micropipette for injection.

Photography intact. To obtain an ideal preparation, both surfaces of the muscle must be cleared of all adherent connective tissue by careful dissection.

The animal was transferred to a board with a glass plate arranged to permit direct transillumination (fig. 1). Cemented to the glass plate is a thin-walled nylon cylinder which serves as a viewing chamber. The tissue was draped over the top of this chamber and held in place by securing the ligatures.

The surface of the exposed muscle was irrigated by a continuous drip of a modified Ringer solution containing 1 per cent oxypolygelatin.8 The

*Oxypolygelatin was generously contributed by Don Baxter, Inc., Glendale, California.

FIGURE 2
Schematic diagram of apparatus used for temperature control of the irrigating fluid: reservoir (R); heater (H); voltage regulator (V.R.); thermoregulator (Th R); thermometer (Th T); viewing chamber (V.C.); thermistors (Th-1 and Th-2).

Temperature of the bathing fluid at the point where it dripped onto the muscle was estimated by a thermistor thermometer. Thermostatic control was by means of a similar thermistor in the outflow system and an appropriate thermoregulator and heating element (fig. 2).

An ordinary binocular microscope was used. A microspectrophotometer was placed in one ocular tube leaving the other available for visual observations. Use of a 50x water immersion objective provided adequate magnification and circumvented the accumulation of condensate on the lens. By means of a Chambers micromanipulator, the tip of a microneedle containing 4 per cent water blue
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TABLE 1
Summary of Mean Clearance Rates as Measured and After Correction for Apparent Viscosity Changes at Various Drip Temperatures

<table>
<thead>
<tr>
<th>TC (°C)</th>
<th>Number of experiments</th>
<th>K_i (min⁻¹) Mean</th>
<th>S.D.</th>
<th>K_i (min⁻¹) corrected</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>5</td>
<td>0.0085</td>
<td>0.0013</td>
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<td>0.0006</td>
<td>0.0136</td>
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<td>29</td>
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<td>0.0120</td>
<td>0.0003</td>
<td>0.0156</td>
</tr>
<tr>
<td>31</td>
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<td>0.0140</td>
<td>0.0010</td>
<td>0.0171</td>
</tr>
<tr>
<td>33</td>
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<td>0.0100</td>
<td>0.0020</td>
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<td>39</td>
<td>12</td>
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<td>0.0089</td>
<td>0.1000</td>
</tr>
<tr>
<td>41</td>
<td>11</td>
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<td>0.0130</td>
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</tr>
<tr>
<td>43</td>
<td>5</td>
<td>0.2358</td>
<td>0.0096</td>
<td>0.1987</td>
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<tr>
<td>45</td>
<td>5</td>
<td>0.2800</td>
<td>0.0154</td>
<td>0.2430</td>
</tr>
</tbody>
</table>

*Mean clearance rate measured at T C.
†Mean clearance rate corrected for viscosity.

was introduced into the muscle slightly to one side of the area from which clearance was to be measured. About 0.04 to 0.06 μl of the dye was injected using minimal pressure. The light transmitted through the injected area was measured by a photomultiplier, the output of which passes through a vacuum tube voltmeter, and was recorded by an Esterline-Angus galvanometer (cf. reference 3). Recordings were taken for a period of 10 to 15 minutes after injection.

With appropriate corrections, the light transmitted can be converted to values representing the concentrations of dye remaining at the injection site. The logarithms of the final corrected values were plotted against time and the calculated slopes, exponential disappearance rates, measured the rate of dye clearance.

Clearances were measured at drip temperatures ranging from 25 to 45 C. with 2-degree increments. The drip temperature was adjusted by resetting the thermostatic control. In a series of special trials, a thermistor placed on the lower surface of the muscle rapidly stabilized within 0.50 to 0.75 C. of the drip temperature. Estimates of capillary flow were made during stabilization of temperature and at intervals during the recording period. At least two determinations of control clearance rate (37 C.) were taken on each animal, and whenever possible, an attempt was made to subject each preparation to temperatures below and above the control. A different site was selected for each injection. It is evident that surgical exposure of the muscle interferes with the homeostatic integrity of the tissue. However, our preparations were reasonably stable when certain requirements were satisfied. These requirements have been extensively described by Zweifach and Metz for the rat mesoappendix preparation and proved to be applicable to the spinotrapezius preparation. Preparations tested at termination of the experiment showed normal vasoactive response to topically applied epinephrine.

Results

The effects of local temperature on the clearance rate of water blue from microinjected sites in living muscle are shown in table 1. Control values obtained in 36 experiments on 18 rats averaged 0.0533 ± 0.0112 min⁻¹. Visual estimates of blood flow at the various temperatures correlated with corresponding clearance rates. The values for clearance after an appropriate viscosity correction* are included in column five of the table.

*The clearance rates were corrected for viscosity changes by the following equations:

\[ K_r = K_i - \frac{K_i \cdot V_3}{V_2} \] (1)

for rates above 37 C. and

\[ K_r = K_i + \frac{K_i \cdot V_3}{V_2} \] (2)

for rates below 37 C., where \( K_r \), \( K_i \), and \( V_2 \) refer to: clearance rates after correction, as measured at T C. and 37 C., respectively; \( V_3 \) and \( V_2 \) are the apparent viscosities of blood at T C. and 37 C. taken from data given by Langstroth.*
table ($K_c$). A more quantitative description of the response to temperature can be gained from the Arrhenius plot of the mean corrected clearance values (fig. 3). A clear break in this relationship occurs at 31°C, with a $\mu$ value of $2.1 \times 10^4$ for the higher temperature range and $0.7 \times 10^4$ for the lower range.

**Discussion**

The microclearance technique can be used to evaluate effective blood flow in skeletal muscle. Direct visual estimates of capillary flow, simultaneous with measurement of clearance rate, give qualitative confirmation of the validity of the clearance measurement. It also demonstrates that the mechanical consequences of intramuscular injection do not disturb the character of the microcirculation: no distortion of tissue or blood vessels at the injection site could be detected. Finally, this method allows injection into consistent or uniform tissue areas.

Water Blue is cleared from microinjection sites in living rat skeletal muscle at 37°C at 0.0533 min$^{-1}$. This compares with clearance from gross intramuscular injections of Na$^{24}$ in man,$^{10}$ 0.05 min$^{-1}$; or dogs,$^{11}$ 0.088 min$^{-1}$; or rats,$^{12}$ 0.05 min$^{-1}$ for Na$^{24}$ and 0.07 min$^{-1}$ for I$^{131}$; and I$^{131}$ in man,$^{5}$ 0.06 min$^{-1}$. Warner et al.$^{5}$ and Bauer et al.$^{13}$ showed significantly more rapid clearance from small injected depots than from 1.0-ml. depots. The agreement between the clearance rates from depot volumes of 0.05 to 0.06 µl used in this study and from macrodepots (all under 0.25 ml) previously noted can be explained only if the inverse relation between clearance and injected volume breaks down below some limiting volume. Agreement between microclearance rates for this dye and gross clearance rates for ions from skeletal muscle lends support to the earlier tentative conclusion that effective blood flow in mesentery$^{2}$ is much more rapid than in muscle.

Data on the relationship between clearance rate and local temperature in skeletal muscle suggest an interesting approach to the analysis of the mechanisms of thermal dilatation. Effective blood flow may be altered as a result of: (1) a change in the apparent viscosity of the blood, (2) a vasodilatation secondary to a change in concentration of metabolites brought about by the local temperature change, or (3) a direct influence of temperature on the smooth muscle elements which determine local resistance.

Although several authors$^{14}$ maintain viscosity changes are primarily responsible for the altered blood flow resulting from a change in temperature, other workers feel that viscosity plays only a minor role.$^{15}$ In any case, this factor must be considered. The temperature-induced changes in clearance observed in this study were considerably greater than the increase in effective blood flow predicted from the thermal viscosity change. That alterations in local blood flow might be secondary to temperature-induced changes in metabolic activity is indicated by Abramson et al.$^{10}$ who showed that direct application of heat or cold produces a significant rise or fall in the oxygen consumption of skeletal muscle in the human forearm. Since the changes in oxygen consumption observed were less than the increases in local blood flow, one must conclude that temperature changes can directly alter the peripheral resistance.

Hyman and his co-workers$^{5,17}$ suggest that tissue clearance might be taken as an index of the "nutritional" flow alone, while plethysmographic measurements of total blood flow would include "shunt" flows. Thus, the data of several authors,$^{10,18}$ who found clear changes in muscle blood flow over the entire physiological temperature range, might be explained by specific opening of shunts due to increase in temperature. In our present study, the increase in clearance rates clearly indicates increase in nutritional flow with an increase in temperature. From the Arrhenius plot, a definite quantitative relationship between clearance rate and local temperature was established. Figure 3 also includes a plot of human forearm muscle flow versus temperature based on the data of Barcroft et al.$^{18}$ corrected to "muscle flow" by the equation of Cooper et al.$^{10}$ and adjusted for viscosity changes (cf. footnote, p. 91). The calculated temperature coefficient for human fore-
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arm muscle flow, over the range 34 to 37 C., 2.0 X 10^4, is similar to that which we obtained between 31 and 45 C, viz., 2.1 X 10^4. There is a significantly lower temperature coefficient for clearance, 0.7 X 10^4 below 31 C. According to established theories,20 the kind of Arrhenius plot obtained suggests that the clearance rate is determined by one master reaction at temperatures between 31 and 45 C. and another for the range between 25 and 31 C.

Summary

The influence of local temperature changes on the effective blood flow in the rat spinotrapezius muscle was investigated by the extension of the microtissue clearance technique originally used for frog mesentery. The clearance of water blue from microinjection sites in rat skeletal muscle at 37 C. agrees favorably with the clearance of other crystalloid labels from gross injection sites in skeletal muscle of mammalian forms. The data show a direct relationship between clearance rates and temperature over a range of 25 to 45 C. The μ value for clearance agrees with the μ value computed from forearm muscle blood flow measurements, at corresponding temperature ranges.

References

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