Validity of the Xenon$^{133}$ Method for Measurement of Muscle Blood Flow Evaluated by Simultaneous Venous Occlusion Plethysmography: 

OBSERVATIONS IN THE CALF OF NORMAL MAN AND IN PATIENTS WITH OCCLUSIVE VASCULAR DISEASE

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Intramuscular injection of a small volume of saline containing radioactive, sodium (Na$^{14}$) was used by Kety for assessing local muscle blood flow.$^1$ With Xe$^{133}$, a radioactive isotope of the lipophilic inert gas xenon, diffusion equilibrium is better maintained than with the hydrophilic Na$^{14}$ ions,$^2$ and Xe$^{133}$ clearance studies allow the estimation of local muscle blood flow per unit weight of muscle, e.g., in ml/100 g/min.$^3$ In order to evaluate in more detail the validity of the Xe$^{133}$ clearance method it was compared to classical venous occlusion plethysmography, both methods being applied simultaneously to the calf of human subjects with and without signs of occlusive vascular disease.

Methods

Xe$^{133}$ dissolved in a concentration of 1 mc/ml in 0.9% NaCl containing no bacteriostatic agent was supplied from Amersham, England. Two intramuscular injections of 0.2 ml were made in each leg studied, one in the anterior tibial muscle and one in the lateral belly of the gastrocnemius muscle. Two scintillation probes (diameter 2 inches), collimated to record from an area of about 10 cm in diameter, were used to record from the two sites.

The probes recorded the radiation that penetrated through the metal container and the water of the plethysmograph. This caused a considerable absorption of the gamma radiation and in several cases it was necessary to inject two or three volumes of 0.22 ml Xe$^{133}$-saline at adjacent points in each of the two muscles to be studied in order to obtain a suitable initial counting rate of $10^5$ counts/min. Due to the fairly large counting field used for both scintillation probes, some degree of overlapping between them was present, but as the plethysmographic observation is an integration of the flow of all tissues of the calf, this overlapping was not considered undesirable.

Both scintillation probes were coupled to a linear precision ratemeter with a time constant of three seconds and the output from the ratemeter was recorded on a logarithmic potentiometer writing on linear paper. The muscle blood flow, $MBF_{xe}$ in ml/100 g/min was calculated by applying the Fick principle to the injected tissue area. Expressing the result per 100 grams of tissue, one obtains:

$$100 \frac{dC}{dt} = MBF_{xe} (Ca-Cv)$$

(1)

where $dC/dt$ is the rate of change of the Xe$^{133}$ concentration, C, (in $\mu$C/g). $Ca$ is the arterial concentration of Xe$^{133}$ which is practically zero as recirculation of the indicator is negligible due to dilution of the tracer in the body and to its rapid elimination via the lungs. $Cv$ is the venous Xe$^{133}$ concentration (in $\mu$C/ml). Assuming that diffusion equilibrium has in all situations been reached between the tissue and the capillary blood leaving it, then $Co = 1/\lambda \cdot C$ where $\lambda$ is the tissue: blood partition coefficient. As $\lambda$ has been determined experimentally to 0.70,$^4,5$ one can solve equation 1 for $MBF_{xe}$:

$$MBF_{xe} = 70 \cdot \left( \frac{- \frac{dC}{dt}}{C} \right)$$

$$= 70 \cdot \left( \frac{-d \log_e C}{dt} \right)$$

$$= 161 \cdot D \text{ ml/100 g/min}$$

(2)

where $D$ is the fraction of one decade that the tangent to the curve decreases in one minute. Using equation 2 the $MBF_{xe}$ can be calculated at any time during the Xe$^{133}$ clearance study.

The venous occlusion plethysmograph used was a segmental (17 cm) waterfilled plethysmograph as described by Folkow$^6$ with some mod-

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The combined venous collecting and arterial compression cuff, 10 cm wide, was placed just above the knee. Another pressure cuff just distal to the plethysmograph was inflated to well above the systolic blood pressure during all measurements.

The measurements were started a few minutes after the injection of the isotope. The venous collecting pressure was 50 mm Hg, and the temperature of the water bath was 34°C in all the studies, while the room temperature was about 25°C. The results of the plethysmographic measurements were obtained in ml/100 ml/min. But, considering the specific gravity of the calf is to be sufficiently close to unity, these results were taken to indicate the calf blood flow/100 g of tissue without the use of any corrections.

After recording the blood flow at rest with both methods, the pressure in the cuff above the knee was suddenly increased to about 300 mm Hg. In some studies circulatory arrest was maintained for one to five minutes without muscular exercise. In other observations the subject performed muscular contractions during the ischemia using both the tibialis anterior and the gastrocnemius muscle, until after about two minutes further, movements were impossible because of pain and muscular weakness. In both types of studies the arterial compression was released suddenly and the blood flow measured for several minutes during the subsequent hyperemia.

The study comprised observations in normal legs and in legs with occlusive arterial disease. The normal controls were obtained in legs of healthy subjects without clinical symptoms suggestive of arterial disease and with normal peripheral pulsations. The patients with occlusive arterial disease had symptoms of intermittent claudication and absent pulsations distally.

### Results

**Resting Values.** The blood flow values obtained at rest are given in table 1. The xenon$^{133}$ method showed about the same resting $MBF_{Xe}$ in both m. tibialis anterior and m. gastrocnemius, and there was no significant difference between the values in the six pairs of observations in normal legs and the seven pairs of observations in legs with peripheral vascular disease. The average resting $MBF_{Xe}$ of all the twenty-five observations was 1.6 ml/100 g/min.

In all studies the plethysmographically recorded calf blood flow was higher than the average of the two $Xe^{133}$ values. There was a tendency towards slightly higher resting values in the group of patients with peripheral vascular disease than in the normal group, but this difference was not statistically significant. The average total resting calf blood flow

### Table 1

<table>
<thead>
<tr>
<th>Study no.</th>
<th>Age</th>
<th>Tibialis m.</th>
<th>Gastrocnemius m.</th>
<th>Plethysmography</th>
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<td>39</td>
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<td>2.8</td>
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<tr>
<td>Mean</td>
<td>29</td>
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<tr>
<td>Occlusive arterial disease</td>
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<td></td>
<td></td>
</tr>
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<tr>
<td>Mean</td>
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<td>1.1</td>
<td>3.1</td>
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</table>

*Same subject as study no. 7 (studies 7 and 8 on the same side).
†Same subject as study no. 11, but studied on the other leg.
flow of all thirteen observations was 3.0 ml/100 g/min.

**Reactive Hyperemia Following a Brief Period of Circulatory Arrest**

After one minute of circulatory arrest only a small and transitory increase of the slope of the Xe$^{133}$ curve was found. A precise evaluation of this change in slope is not possible due to the inherent statistical fluctuations of the counting rate, but for both Xe$^{133}$ injection sites the cumulative blood flow during the first minute following release of the cuff, approximately doubled and then returned to resting level. The plethysmographic curve showed a hyperemic response after a 1-minute occlusion with a brief peak blood flow value of 15 ml/100 g/min, the cumulative flow over the first minute also amounted to about twice the resting value.

When the circulatory arrest was prolonged three to five minutes, the ensuing hyperemia was well demonstrated by the Xe$^{133}$ curves. Also in these studies the Xe$^{133}$ flow values were lower than the plethysmographic values during the first 10 to 20 seconds. After this initial period the flow curves were quite similar (fig. 1).

In four of the above-mentioned studies the time constant of the ratemeter was changed from three to one second without changing the result. This means the initial slowness of the MBF$_{Xe}$ response to a brief period of circulatory arrest was not due to a damping effect of the ratemeter circuit.

**Hyperemia Following Muscle Work During a Brief Period of Arrested Arterial Inflow**

Eight observations were made on normal legs (figs. 2 and 3, and table 2). In all these

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**FIGURE 1**

Muscle blood flow in ml/100 g/min evaluated simultaneously by Xe$^{133}$ clearance in the anterior tibial muscle, in the gastrocnemius muscle, and by venous occlusion plethysmography. Study was made after five minutes ischemia without muscle work in a normal man aged 25 years.

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**FIGURE 2**

Hyperemia study no. 4 in a normal leg of hyperemia following muscle work during ischemia. See legend figure 1.

**FIGURE 3**

Hyperemia study no. 5, same as in figure 2, in a normal leg.
TABLE 2
Comparison of Xe\textsuperscript{133} Clearance Method and Venous Occlusion Plethysmography in Eight Normal Legs Studied During Reactive Hyperemia Following Muscle Exercise During Ischemia

<table>
<thead>
<tr>
<th>Hyperemia study no.</th>
<th>Age</th>
<th>Maximal blood flow (ml/100 g/min)</th>
<th>( T_{1/2} ) in minutes for return of the calculated blood flow to the resting level</th>
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</thead>
<tbody>
<tr>
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<td></td>
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<td>Gastrocnemius m.</td>
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<td>2</td>
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<td>3*</td>
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<td>64</td>
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</tr>
<tr>
<td>8</td>
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<tr>
<td>( \bar{x} )</td>
<td>27</td>
<td>48.3</td>
<td>31.5</td>
</tr>
</tbody>
</table>

*Same subject as study no. 2, but studied on the other leg.

\[ \text{Same subject as study no. 6, and studied on the same leg (in study no. 6 special muscular effort of gastrocnemius m. was carried out; in study no. 7, of tibialis anterior m.).} \]

studies the maximal blood flow recorded with the Xe\textsuperscript{133} method was higher over the anterior tibial muscle (avg 48 ml/100 g/min) than over the gastrocnemius muscle (avg 32 ml/100 g/min). In studies no. 6 and 7 the same normal leg was studied with an interval of about one-half hour. By instructing the patient to exercise mainly m. gastrocnemius (study no. 6) and m. tibialis anterior (study no. 7) it was found possible to alter somewhat the maximal flows recorded by the Xe\textsuperscript{133} method for these two muscles.

The plethysmographic values exceeded the Xe\textsuperscript{133} values during the first 15 seconds after release of the cuff (figs. 2 and 3). During the remainder of the hyperemic reaction the results of the two methods were in good agreement. The maximal calf blood flow averaged 42 ml/100 g/min and was usually found to lie between the corresponding Xe\textsuperscript{133} values. After maximal blood flow had been observed it took on the average 0.8 minutes for all three blood flow curves to reach half-way down toward the resting level.

Eight observations were made on legs with occlusive arterial disease as manifested by intermittent claudication (figs. 4 and 5, and table 3). In these observations the maximal MBF\textsubscript{Xe} values were all below those found in the normal group, again with the MBF\textsubscript{Xe} values recorded over the anterior tibial muscle (avg 15 ml/100 g/min) significantly exceed-

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**FIGURE 4**
Hyperemia study no. 11, same as in figure 2, in a leg with occlusive arterial disease.

**FIGURE 5**
Hyperemia study no. 13, same as in figure 2, in a leg with occlusive arterial disease.
VALIDITY OF XE$^{133}$ MUSCLE BLOOD FLOW METHOD

TABLE 3
Comparison of Xe$^{133}$ Clearance Method and Venous Occlusion Plethysmography in Eight Legs with Occlusive Arterial Disease: Maximal Blood Flow During Reactive Hyperemia Following Muscle Exercise During Ischemia

<table>
<thead>
<tr>
<th>Study no.</th>
<th>Maximal blood flow (ml/100 g/min)</th>
<th>Tibialis m.</th>
<th>Gastrocnemius m.</th>
<th>Plethysmography</th>
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<tr>
<td><strong>x</strong></td>
<td>15.1</td>
<td>11.3</td>
<td>23.4</td>
<td></td>
</tr>
</tbody>
</table>

*Same subject as study no. 9 (studies 9, 10, and 11 on the same side).
†Same subject as study no. 14, but studied on the other leg.

ing those recorded over the gastrocnemius muscle (avg 11 ml/100 g/min).

In the diseased legs plethysmographically measured maximal calf blood flow was also reduced (avg 23 ml/100 g/min) below the level in the normal group. In all but one instance, however, the plethysmographic value was higher than the highest Xe$^{133}$ value. There was not as marked an initial discrepancy between the two methods as in the normal group. The return to resting blood flow values was so slow in this group that its half-time of return to resting level could not be accurately assessed.

**Discussion**

Muscle blood flow can be assumed to be the major component of calf blood flow during maximal reactive hyperemia in normal man. For this reason, the agreement of the two methods during maximal reactive hyperemia in normal subjects points to the validity of the Xe$^{133}$ method. Thus, both the use of 0.7 as the value for the tissue: blood partition coefficient and the fundamental assumption of an essentially maintained diffusion equilibrium for Xe$^{133}$ between the muscle tissue and its capillary blood even during maximal blood flow have found direct experimental support. This agreement also implies that the unavoidable local trauma caused by the injection plays a relatively minor role with the technique employed here. We shall not comment further therefore, in the remaining part of the discussion, on this point and will take the results to indicate what the two methods may measure. The Xe$^{133}$ method measures the outflow of nutritive blood from the injected muscle area per unit weight (100 g) of tissue. The plethysmograph measures total arterial inflow per unit volume (100 ml) of the calf segment which, as mentioned, can be taken to equal the inflow per unit weight (100 g).

Total resting blood flow of the calf averaged 3.0 ml/100 g/min, whereas the simultaneously measured resting nutritive muscle blood flow averaged 1.6 ml/100 g/min. A high skin blood flow at a room temperature of 25°C could, perhaps, account for this difference. Unpublished observations by Andrée Larsen, Quaade, and Lassen have shown that subcutaneous tissue blood flow exceeds resting muscle blood flow. It appears likely, therefore, that the blood flow through non-muscle tissue can account for the observed difference between the resting values of the two methods, i.e., that shunt flow is minimal in the resting skeletal muscle.

During hyperemia there is, just after release of the occluding pressure, an unsteady state of blood flow as inflow into the calf segment exceeds the simultaneous venous outflow. In all cases there is a shift of the base-
line of the plethysmograph indicating an acute increase in calf volume of about 0.5%.
This could account, in a qualitative sense at least, for the observed discrepancy of the two methods during the initial 15 seconds of the hyperemic response. The cumulative flow during the first minute of simple reactive hyperemia shows approximately a doubling of the resting flow value for both methods. This suggests that all the tissues of the calf participate in this hyperemia.

After ischemic work in normal man the similarity of the general shape and amplitude of the hyperemic response, as measured by the two methods, has already been commented on. This similarity excludes gross shunting of blood in the muscles or in the other calf tissues during maximal blood flow after ischemic exercise. In the patients with intermittent claudication the present small series showed a higher maximal blood flow through the total calf than through the two muscles examined. This may have several explanations. The most likely one appears to be that perfusion of muscle is uneven in such patients. The proximal parts of the calf muscles may receive a relatively more ample perfusion than those parts of the muscle used for measurements. In addition, the contribution from skin and subcutaneous tissue may be more important quantitatively in such patients than in normal man where muscle blood flow dominates. The possibility that the tissue: blood partition coefficient is altered in muscles subjected to chronic ischemia seems relatively unlikely.

We have not, in the present study, stressed the diagnostic value of the Xe\textsuperscript{133} studies in intermittent claudication. In such patients the reactive hyperemia following maximal work during ischemia is subnormal and has a delayed onset. This test is of considerable diagnostic value and it is used routinely for clinical work in our hospitals. The test is easy to perform, it demands no standardization, and the result is available immediately after the study. In these respects the Xe\textsuperscript{133} method compares favorably with the plethysmographic method.

The values for maximal blood flow after ischemic exercise suggest that with this test the Xe\textsuperscript{133} technique may even be more sensitive than venous occlusion plethysmography for discriminating between normal and obstructed arterial supply to the muscles. This possibility can only be evaluated by quite extensive comparative studies. Such studies lie, however, beyond the scope of this investigation which was aimed primarily at examining the validity of the Xe\textsuperscript{133} modification of Kety's local clearance method.

**Summary**

Calf blood flow has been measured simultaneously by the Xe\textsuperscript{133} clearance method in m. tibialis anterior and m. gastrocnemius and by venous occlusion plethysmography in normal man and in patients with intermittent claudication.

At rest Xe\textsuperscript{133} muscle blood flow (MBF\textsubscript{Xe}) averaged 1.6 ml/100 g/min while plethysmographic total calf blood flow (calf BF) was higher in each instance and averaged 3.0 ml/100 g/min. There was no difference between normal legs and legs with occlusive vascular disease in this respect.

Following one to five minutes of arterial occlusion, reactive hyperemia was seen with both methods, the rapid changes being most readily observed with the plethysmographic method.

After exhaustive muscular exercise during ischemia in normal man maximal MBF\textsubscript{Xe} averaged 40 ml/100 g/min (48 ml/100 g/min over m. tibialis anterior and 32 ml/100 g/min over m. gastrocnemius). The maximal calf BF averaged 42 ml/100 g/min. The rate of return of blood flow to the resting level was also quite similar with both methods. In the patients with claudication both methods showed a subnormal and delayed hyperemic reaction following ischemic work, but the MBF\textsubscript{Xe} was consistently lower.

The results obtained indicate that the Xe\textsuperscript{133} modification of Kety's local clearance method for measurement of muscular blood flow in man is essentially valid.

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References


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