Inert Gas Diffusion Method for Measurement of Blood Flow Using Saturation Techniques

COMPARISON WITH DIRECTLY MEASURED BLOOD FLOW IN ISOLATED GASTROCNEMIUS MUSCLE OF THE CAT

By Per Sejrsen and Knud H. Tønnesen

ABSTRACT

The isolated gastrocnemius muscle of the cat was used to study the relation between the desaturation of $^{133}$Xenon measured by external monitoring, and blood flow measured directly by a dropcounter.

The metered blood flow agreed with that calculated from the initial slope of the desaturation curve from tissue equilibrated by prolonged intra-arterial infusion and also by atraumatic gas labelling of a local area of the muscle (approximately 0.15% of the muscle weight). In this sense the concept of uniform distribution of blood flow in skeletal muscle has received substantial support. The identity of the results obtained by local gas labelling and by intra-arterial equilibration of the whole muscle with gas implies that there is, at least initially, diffusion equilibrium between tissue and blood in both.

However, the clearance curves could in no instance be fitted by a single exponential function. This indicates that the diffusion equilibrium is not maintained after the initial phase. It is suggested that this is not due to the presence of anatomical arteriovenous shunts or other types of uneven perfusion, but to counter current exchange of inert gas through the walls of vessels, i.e. shunting by diffusion explains the shape of the desaturation curves.

ADDITIONAL KEY WORDS

residue detection of $^{133}$Xenon
intra-arterial step input
direct recording of blood flow
uniform distribution of blood flow in skeletal muscle

input should theoretically be based on the initial washout rate of the desaturation curve (2), whereas in the bolus injection experiment the total area under the washout curve (when the height is unity) is utilized (3). However, from further theoretical considerations to be given in this paper it was found that the stochastic equation is inadequate to yield a useful equation for calculating mean blood flow after intra-arterial step input. Only by assuming diffusion equilibrium between tissue and blood in an initial time interval was it possible to obtain a useful equation. The purpose of the experiments using intra-arterial step input will then be to validate experimentally the assumption of diffusion equilibrium in an initial time interval.

A third mode of labelling a tissue is that of applying the tracer locally (4-6). This

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widely used technique necessitates that the tissue is equilibrated in the same fashion as by the intra-arterial step input. Of considerable practical importance are two more problems which are not related to the theory of calculation: the labelling procedure must not alter the circulation locally, and the local blood flow must be representative of that of the entire tissue. The purpose of the local labelling experiments will then be to examine these three conditions. To do this it was necessary to develop an atraumatic technique for local labelling of tissue.

The experiments were carried out in the autoperfused isolated gastrocnemius muscle of the cat, and direct venous outflow was recorded. The different possibilities of explaining the shape in a semilog diagram of the observed desaturation curves will be considered.

Theoretical Considerations

THE PARTITION COEFFICIENT

When a tissue in the steady state is saturated by intra-arterial step input (i.e. by a constant arterial concentration, $C_{\text{blood}}$), then the tracer concentration will rise towards equilibrium in the relative volume of distribution for the tracer in question. At saturation, the ratio of the concentrations of the tracer in the tissue $C_{\text{tissue}}$ and in the blood is given by the partition coefficient $\lambda$:

$$\lambda = \frac{C_{\text{tissue}}}{C_{\text{blood}}} \text{ ml/g.}$$

(1)

The tissue concentration is measured in counts/min per g of tissue, and the concentration in the blood in counts/min per ml of blood. Therefore $\lambda$ has the unit of ml of blood/g of tissue, and $\lambda$ is thus the relative volume of distribution.

In this definition the amount of tracer in the blood within the tissue is included in $C_{\text{tissue}}$. In fact, for intravascular tracers the whole amount of tracer in the tissue is contained within the blood vessels. For this type of tracer the vascular volume therefore equals the volume of distribution. However, for diffusible tracers this volume of distribution is considerably larger.

STOCHASTIC ANALYSIS

In the present experiments equilibrium of the muscle tissue with $^{133}$Xenon was taken to be fulfilled, when no significant increase of the count-figures was observed by external monitoring.

During desaturation, the loss of tracer ($-dq$), in a short period of time ($dt$), is the product of the time interval, the blood flow rate ($F$), and the concentration of tracer in the blood ($C_{\text{blood}}$) if there is no recirculation. Thus:

$$-dq = dt \cdot F \cdot C_{\text{blood}}$$

(2)

Using residue detection only (see experimental procedure), $C_{\text{blood}}$ must be eliminated. The tissue has been equilibrated to the blood. Therefore it is valid to insert equation 1 into 2 at the start of the desaturation process:

$$-dq = dt \cdot \frac{FC_{\text{tissue}}}{\lambda}$$

(3)

$$t \to 0.$$

Multiplying the numerator and denominator on the right side by the weight of the tissue ($W$) and rearranging one obtains:

$$-\frac{dq}{dt} = \frac{F}{W} \cdot \frac{q_{\text{tissue}}}{\lambda}$$

(4)

$t \to 0,$

where $q_{\text{tissue}}$ is the amount of tracer in the tissue at time infinity of the saturation process.

Defining $f$ as the mean blood flow/100 g of tissue, one obtains:

$$f = 100 \cdot \lambda \cdot \frac{-dq/dt}{q_{\text{tissue}}} = 100 \cdot \lambda \cdot 1/l \text{ ml/100 g \cdot min,}$$

(5)

i.e. the blood flow/unit weight equals $\lambda$ multiplied by the reciprocal value of the time constant ($l$) at time zero of the desaturation process. The time constant at time zero is denoted $t$ because it is the weighted mean

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value of all time constants in the washout process (2) (i.e., the mean transit time of the indicator gas through the muscle). t is obtained as the time from the interruption of the infusion to the intercept of the slope with the time axis (Fig. 1A).

The stochastic analysis outlined here for calculating mean blood flow after complete

A. Linearly plotted curve obtained by external monitoring of the isolated gastrocnemius muscle after intra-arterial step input. Although the blood flow was constant throughout the experiment, the time constant is longer for the saturation than for the desaturation process. This is because the input was convoluted over the volume of the catheter during the saturation.

B. Semilogarithmic plot of the curve in Figure 1A. It is seen that the desaturation curve cannot be described by a single exponential.
saturation is based on Zierler's concepts and has been discussed theoretically by Lassen (2). The assumptions on which it is based are listed below; they are, of course, intimately related to those of the stochastic equations for blood flow calculation after bolus injection (3, 1).

1. Equivalent entry, i.e. the tracer particles are completely mixed with the blood at the entrance to the organ.

2. Stationarity, i.e. that the distribution of transit times does not change with time, which implies that the total blood flow is constant. For intra-arterial step input this is necessary during the saturation process. For unit input it is necessary during the washout process.

3. Isoefficiency, i.e. that there is equal counting efficiency for all parts of the muscle. The half thickness value of self absorption for $^{133}$xenon $\gamma$-radiation in muscle tissue is not negligible (1). Therefore isoefficiency in this context implies that all tissue elements must be represented equally in all depth of the tissue.

4. The recirculation must be negligible or known.

5. The tracer must not leave the organ under consideration by diffusion from the surface.

The derivation does not imply rapid diffusion equilibrium and hence it is also valid for intravascular ("nondiffusible") indicators.

From this theory it is obvious that the outflow of fully saturated blood will only last as long as the shortest vascular transit. From equation 5 it appears that this initial washout is not an exponential but a linear process, because all terms in the equation are constant for a short but finite time interval. This means that the clearance curve plotted in a linear diagram will have a linear initial part while the same curve plotted in a semi-logarithmic diagram will have a slightly increasing slope in this initial phase. However, for the reasons discussed below the calculation of the blood flow from this stochastic analysis is invalid for diffusible indicators. The shortest transit times measured for $^{133}$xenon ranged from 6 to 20 seconds (1).

At the time of interruption of the infusion, the mixing in the artery of labelled and unlabelled blood (viz, convolution over the arterial volume from the tip of the catheter till the measuring field) will exclude evaluation of the mean transit time from the first few seconds of the desaturation process. The duration of the peak plateau in the bolus experiments (1) was therefore only 4 to 6 seconds while the shortest measured transit times were 6 to 20 seconds. It can then be expected that in the intra-arterial step input experiments the "initial linear slope" will also last only 4 to 6 seconds. As $^{133}$xenon has a volume of distribution about eighteen times greater than the vascular volume, the initial decrease of activity will be only a small fraction (from 4 to 0.4%) of the initial height of the curve. This short interval available for measuring the slope and the small decrease of the activity, invalidates any exact determination of the "initial linear slope" for $^{133}$xenon simply due to counting statistics. For an intravascular tracer such as RISA this stochastic analysis may be possible, because the decrease of activity within the shortest time of transit will be a much larger fraction of the initial amount of tracer.

Thus it must be concluded that the stochastic analysis for a tissue equilibrated with a freely diffusible tracer is unsuitable for calculation of the mean blood flow after continuous infusion (saturation). This is at variance with the conditions after bolus injection (1). The difference is caused by the fact that the height of the bolus curve can be determined adequately from the count-figures during 4 to 6 seconds while determination of the initial slope of the desaturation curve needs to be recorded for a considerably longer time interval to obtain a similar degree of accuracy.

The above considerations imply that a quite different approach must be made to calculate the mean blood flow from desaturation curves after intra-arterial step input for freely diffusible tracers.

**EXponential analysis**

Utilizing the assumption first proposed by
Zuntz (7) and later by Kety of maintenance of diffusion equilibrium between the tissue and its effluent venous blood, the clearance curve should be monoexponential throughout its course,

\[ q_{\text{tissue}}(t) = q_{\text{tissue}}^0 \exp\left(-\frac{t}{\lambda t}\right), \quad (6) \]

where \( q_{\text{tissue}}(t) \) is the amount of tracer at time \( t \), and \( \lambda \) is the blood flow/g * min (2, 4).

To use this equation to calculate the blood flow from the clearance curve it is not necessary that diffusion equilibrium is maintained throughout the washout process. Assume that during some time interval in the beginning of the desaturation process a high degree of equilibrium exists; if this time interval is sufficiently long to secure an evaluation of the monoexponential clearance rate, then it is possible to calculate the blood flow. From the previous study (1) it was observed by external recording that the washout of \(^{133}\text{Xe}\) non from a bolus input to skeletal muscle does not follow a single exponential throughout its course. The main purpose of the step input experiments in the present study is then to evaluate whether an approximated initial, monoexponential slope of the curve in a semi-logarithmic diagram is related to the blood flow, i.e. to evaluate whether diffusion equilibrium between tissue and blood is practically maintained in this initial time interval.

The equation for calculating the blood flow is then obtained by solving equation 6 for \( \lambda \):

\[ \lambda = \frac{dq/dt}{q(t)_{\text{tissue}}} \quad (7) \]

\[ \int = 100 \times 10^{-1} \text{ ml/100 g} \text{ min} \]

for \( t \leq \Delta t \).

The concentration of tracer in the venous blood at a certain time was equilibrated with the tissue in the capillaries at a short time interval earlier, when the tissue concentration was slightly higher. Therefore the venous blood may contain a slightly higher concentration than the mean tissue concentration (calculated to be maximally about 2%). This will yield a slightly faster clearance rate. In contrast, the blood in the capillaries may not be quite equilibrated with the tissue. This will yield a clearance rate that is too slow. The latter point is of some importance since it is theoretically impossible that equilibration is truly complete at any time after the onset of desaturation.

**LOCAL EQUILIBRATION**

If blood flow in the whole muscle is to be calculated from the approximated initial monoexponential part of the desaturation curve obtained from a local atraumatically labelled area, Kety's concept must be extended by the following assumptions:

1. That the blood flow is uniformly distributed, i.e. that blood flow in the fraction of the muscle which is labelled, is representative for the whole muscle.
2. That the tissue is equilibrated in the same fashion as by the intra-arterial step input.
3. That the continuous diffusion within the muscle tissue during the labelling period and during the desaturation does not invalidate the calculation of the blood flow from the clearance curve (8, 9).

If the blood flow calculated from the desaturation curve from a local depot equals the directly measured venous outflow from the whole muscle then the three assumptions mentioned above are likely to be fulfilled. Furthermore, this will also imply an experimental verification of virtual diffusion equilibrium between tissue and blood at the end of the labelling procedure.
Experimental

THE ISOLATED GASTROCNEMIUS PREPARATION

The isolated gastrocnemius muscle of cats was used as described (1). In this preparation only lean muscle tissue was considered; all visible fat was removed. To obtain blood flow rates at different levels and yet be fairly constant in each experiment, the isolated sciatic nerve was stimulated by silver electrodes using a S4 Grass stimulator. The frequency was 1 to 3/sec, the voltage 6 to 8 v and the duration 1 msec. In three experiments no stimulation was applied. The hemoglobin concentration was measured in each single experiment. To obtain steady blood pressure and hemoglobin concentration all cats were given donor blood.

The carefully shielded scintillation detector "saw" only the muscle tissue proper, and was situated at a distance of 14 cm to eliminate the effect of change of the geometry in the local labelling experiments caused by diffusion of the tracer into deeper layers of the muscle tissue (10). The collimation was so wide that the whole local depot could be seen throughout the experiment. The number of pulses from the scintillation detector were printed out every 2 seconds from a sealer with a memory (Meditronic, Denmark) so that the counts could be recorded with a time loss of only 10 ^isec per period. By using an amplifier/analyzer (Philips PW 4280) only the 81 kev γ-emission was counted. During the whole study the background was closely around 25 counts/min.

The clearance curve was plotted in a semi-logarithmic diagram after subtraction of the background. The T1/2 was determined by entering a straight line by eye at the initial part of the curve. In the step input experiments the initial slope was also calculated by the method of least squares.

The blood pressure was continuously recorded by a Statham (p 23 AA) transducer. The output from the photoelectric drop counter measuring the outflow from the femoral vein was evaluated by an ordinate writer. These parameters were recorded on a Beckman 4-channel Dynograph type R.

SATURATION BY INTRA-ARTERIAL STEP INPUT

In the experiments where the tissue was labelled by an intra-arterial step input, a polyethylene catheter was inserted into a side branch of the femoral artery. The infusion was given from a 10-ml syringe by a constant infusion apparatus. The concentration of 133Xenon in saline was 0.1 to 0.2 mc/ml, (The Radiochemical Centre, Amersham, England).

To avoid the activity within the syringe of infusion being counted by the probe, a rather long catheter (50 cm) was used. To avoid a large resistance in this catheter the inner diameter was 1.0 mm. The volume of the catheter was 0.39 ml. With an infusion rate of 0.2 ml/min, the mean transit time for the catheter was about 2 minutes. Because of this high value the determination of the mean transit time from the slope at the onset of the saturation period is inaccurate. However, the start of the desaturation process was unaffected by the catheter (see Fig. 1A and 1B). In experiments where the blood flow was arrested after intra-arterial saturation, the loss of 133Xenon from the surface of the muscle was found to be negligible in comparison to clearance by blood flow (less than 1%).

LOCAL SATURATION BY DIFFUSION

In the experiments 133Xenon gas was introduced by diffusion into the muscle from the surface. The 133Xenon gas bubble was carefully withdrawn from the ampule to a 1-ml syringe and replaced by saline. A hypodermic needle was inserted into the top of a plexiglass cup (10 mm inner diameter and 1 mm inner height) (Fig. 2). The cup was applied to the moist surface of the muscle and the 133Xenon gas (5 to 10 mc/ml) introduced into the closed chamber, made up by the cup and the muscle surface for 30 seconds and the 133Xenon gas was then redrawn into the syringe. This technique of atraumatic local labelling of tissue was developed from that of Sejrsen (10). After the cup was removed, surplus 133Xenon was blown away and a Mylar film, 20 μ thick and measuring 2.5 by 4 cm, was made to adhere to the moist surface of the muscle.

For all practical purposes, this membrane is impermeable to 133Xenon gas. After the blood flow was brought to the desired level by stimulation of the sciatic nerve, the stimulation was
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**A. Following Intra-Arterial Step Function Input**

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*The standard deviation of the dropcounter flow values minute by minute during the plateau of the saturation curve plus the time during the initial slope.
†Values obtained from the slope fitted by eye.
‡Values obtained by calculation of the slope of the regression line by least squares.

MBF = muscle blood flow; H = maximum height of the curve; t = mean transit time; T½ = half time.
interrupted and the gauze and polyethylene sheet covering the muscle were removed. The labelling was then performed, the muscle was wrapped up again and the stimulation of the sciatic nerve was resumed. This procedure took about 1 minute. Recording of the desaturation curve was then initiated.

It was observed that it is of importance that the cup be applied to the muscle surface without pressure, furthermore that the muscle surface must not be exposed for too long a time to the colder surroundings.

**Partition Coefficient**

The λ values used at different values of hemoglobin concentration have been taken from our earlier study (1). The hematocrit value of 43% was taken to correspond to 14.5 g% of hemoglobin.

**Recirculation**

The recirculation was evaluated in one experiment by giving equal amounts of 133Xenon solution as a step input first into the femoral artery and subsequently intravenously. After 25 minutes of intra-arterial xenon infusion the level of activity was steady as measured by external recording. After complete desaturation the same amount of 133Xenon was then infused intravenously with the same rate. The maximum count-figure after 25 minutes is considered to express the recirculation.

**Results**

**The Intra-Arterial Saturation Experiments**

Simultaneous measurements of venous outflow and 133Xenon desaturation curves after intra-arterial step input were performed in 7 experiments on 7 cats. The results are given in Table 1, A. The muscle weights measured at the end of the experiments ranged from 21.9 to 48.1 g. The contralateral gastrocnemius muscle was also removed and weighed. By comparison it was found that little edema had occurred in the muscle used in these experiments. The blood flow measured by the drop-counter (in ml/min) divided by the muscle weight and multiplied by 100 gives the flow in ml/100 g · min. No correction for the edema was applied in this calculation.

In each experiment the muscle blood flow was calculated from the venous outflow, minute by minute, during the whole experiment. The constancy of the flow is expressed as the standard deviation of the flow recorded in this way. Table 1, A also lists the hemoglobin concentration and corresponding λ values.

In the present experiments, the tissue was considered to be saturated when the count-figures were constant within the statistical range for 6 to 25 minutes. The maximum counting rates obtained were from 1,120-27,000 counts/min.

After interruption of the infusion, the maximum clearance rate, i.e. the exponential slope in the beginning of the curve, was constant for 0.7 to 4.4 minutes. During this time the count-figures decreased from 10 to 62% of the initial value. The desaturation curves were recorded until 0.1 to 2.5% of the initial counting rate was reached, except in one experiment with resting flow and this was discontinued at 49% of the initial counting rate. Both the mean transit time and the corresponding T½ values are listed (T½ = t·ln 2). The t values ranged from 2.4 to 62.6 ml/100 g · min.

The recirculation in one experiment was evaluated to be 1.0% and is not corrected for.

The relation between the directly measured venous outflow and the t values is seen in Fig. 3 (the T½ used was that obtained by the least squares method). The coefficient of
correlation $r = 0.995$, $P < 0.001$; the slope of the regression line $b = 1.045$, $sd$ of $b = 0.049$; the intercept was $-1.9$, $sd$ of the intercept was 1.7 ml/100 g min. By the $t$-test it was demonstrated that the intercept was not significantly different from zero. Assuming the regression line passed through zero, then

$$b' = \frac{\Sigma xy}{\Sigma x^2}$$

was calculated to be 0.998.

**THE LOCAL SATURATION EXPERIMENTS**

Venous outflow and clearance from the area of the muscle, which was labelled from the surface by means of gas diffusion technique, were measured simultaneously in 14 experiments on 11 cats.

The results are given in Table 1, B, which is analogous to Table 1, A from the first series of experiments, and will therefore be commented on only briefly. The maximum count-figures were from 290,000 to 770 counts/min. The duration of the well defined first slope, in which the clearance rate was constant, lasted from 1.0 to 7.0 minutes. The cumulative clearance in this period was from 14 to 84%.

The venous outflow varied between 3.9 and 41.9 ml/100 g min and the $f$ from 3.0 to 41.5 ml/100 g min.

Figure 4 shows the relation between the venous outflow and $f$. The coefficient of correlation $r$ was 0.90, $P < 0.001$. The slope of the regression line $b = 0.90$, $sd$ of $b = 0.13$. The intercept was 2.4 ml/100 g min and $sd$ of the intercept 3.0 ml/100 g min; by the $t$-test it was demonstrated that the intercept was not significantly different from zero. $b'$ was calculated to be 0.98.

**COMPARISON OF THE SHAPE OF THE DESATURATION CURVES AFTER INTRA-ARTERIAL STEP INPUT AND LOCAL GAS LABELLING**

It is difficult to carry out this comparison because only the curves obtained by the same blood flow and the same $\lambda$ can be expected to be congruent as in both types

![Figure 4](http://circres.ahajournals.org/)

**FIGURE 4**

Comparison between the directly measured blood flow and the blood flow calculated from the initial slope of the desaturation curves after local gas-labelling.

**COMPARISON OF THE SHAPE OF THE DESATURATION CURVES AFTER INTRA-ARTERIAL STEP INPUT AND LOCAL GAS LABELLING**

It is difficult to carry out this comparison because only the curves obtained by the same blood flow and the same $\lambda$ can be expected to be congruent as in both types
of experiments the shape of the curve varied with flow. In this comparison there were only two pairs of curves in which the directly metered blood flow matched, where the $\lambda$ values were almost equal, and in which the blood flows were constant for a sufficiently long time for description of the shape of the curves. One of the pairs (Fig. 5) was obtained at resting flows and the other at a flow rate about 30 ml/100 g.min (Fig. 6). It is seen from these figures that the shapes of the curves are practically identical at equal blood flow rate. Also in the remainder of the experiments the shape varies with flow in the same manner in both types of experiments.

The two-compartment-in-parallel-analysis, applied in the previous study using bolus injection (1) could not be used. Only the desaturation curves in experiments with resting flows fitted the two-compartment-in-parallel-model whereas in the large majority of the curves at least three exponentials were necessary to describe the total curve. As the parameters obtained by this analysis were considered to be too arbitrary in relation to the experimental accuracy, this technique of describing the curves was considered unsuitable.

**Discussion**

**Uniform Distribution of Muscle Blood Flow**

The results indicate that the blood flow calculated from the initial monoexponential slope of the desaturation curves of $^{133}$Xenon equals the directly measured flow, regardless of the route by which the tissue has been labelled.

The greater deviation of the results of the local labelling experiments than in the intra-arterial step input experiments, both compared to the directly measured blood flow, must be commented on. In the intra-arterial

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*Figure 6*

Semilogarithmic plot of two desaturation curves from two experiments with exercise blood flow. The crosses denote a curve from an intra-arterial step input experiment and the dots a curve from a local gas-labelling experiment. (The intra-arterial saturation experiment is No. 1, the gas-labelling experiment is No. 13).
step input experiments the desaturation was affected by interruption of the infusion of the $^{133}$xenon solution into the femoral artery. The nerve stimulation was continued and the gauze and plastic sheet covering the muscle were kept in place.

In contrast, the experiments with local gas-labelling involved interruption of the stimulation. Also, the cover over the muscle was removed, exposing it to the colder surroundings for about 1 minute. After labelling, the muscle was again wrapped up and stimulated in order to reestablish the previous level of steady blood flow. The venous outflow was then calculated from the mean value of the dropcounter flow in the period where the initial monoexponential slope was defined.

The greater deviation of the results in the gas-labelling experiments than in the directly measured flow may well be explained by these differences in the experimental procedures.

From these considerations it is concluded that the results of the gas-labelling experiments indicate that the blood flow in the local area is representative of the blood flow in the whole muscle: no evidence for a systematic "higher-than-average" or "lower-than-average" blood flow of the superficial layer of the muscle was found experimentally.

The size of the area labelled by the local gas application technique was evaluated in a separate study (to be published). It was demonstrated that more than 99% of the amount of $^{188}$xenon which had entered into the muscle by diffusion from the surface in 30 seconds was located in a superficial layer of 0.5 mm thickness. As the labelling chamber used was 10 mm in diameter this tissue mass weighed about 50 mg. The total muscle weight was about 30 g, i.e. the labelled fractions were then about 0.15% of the whole muscle.

As demonstrated in the results, two pairs of curves from the intra-arterial step input and the gas labelling experiments could be compared directly because the respective values of blood flow and $\lambda$ matched (Figs. 5 and 6). The congruency of the two types of curves demonstrates that the distribution of washout times for the tracer in a small fraction is the same as that of the whole muscle, i.e. the local gas-labelling mimics a saturation by intra-arterial step input.

Uniform distribution of muscle blood flow in this paper is thus referring to uniformity down to tissue volumes of the above-mentioned size. It is interesting to note that the first assumption on which the theory of the present paper rests, viz, that of equivalent entry, would appear to be unnecessary in the fairly homogeneous skeletal muscle tissue.

**DIFFUSION EQUILIBRIUM BETWEEN TISSUE AND BLOOD**

In the intra-arterial step input experiments the initial monoexponential part of the $^{133}$xenon clearance curves had a duration from 0.7 to 4.4 minutes. The calculated blood flow ranged from 2 to 60 ml/100 g .min and matched the directly measured venous outflow. This indicates, as discussed in the theoretical considerations, that during these time intervals a very high degree of diffusion equilibrium between tissue and blood must exist. The concept of equilibrium implies that not only muscle cells, connective tissue, and interstitial fluid, but also blood within the vessels is saturated. Considering the local gas-labelling experiments then all tissue elements, i.e. also the blood within the exchange vessels, must have been well equilibrated at the end of the application. This is so because the local gas-labelling experiments yielded blood flow values equal to the directly measured flow.

It is evident that with local labelling a concentration gradient exists from the surface into the muscle tissue. However, this will only bring forth new tissue volumes having the same properties as the first labelled ones and each having practically diffusion equilibrium between tissue and blood. This concept which is denoted isotropy has been treated mathematically by Perl (8, 9). We conclude that within an initial time interval, Kety's original assumption of diffusion equilibrium between tissue and blood has been given experimental support.
SHUNTING BY DIFFUSION

The shape of the 133xenon clearance curves in the present study could not be described by a single exponential throughout their entire course. This could be due to the circumstances listed below.

In-Parallel Arrangements

Anatomical Blood Shunt.—If a fraction of the total inflowing blood to the muscle bypasses the exchange vessels then unequilibrated blood will leave the muscle. This blood will naturally also be measured by the drop-counter. This possibility is not a likely one as the initial clearance rate in both types of experiments in the present study equalled the directly measured venous outflow from the whole muscle. The same conclusion was reached in the previously reported bolus injection studies (1). The throughfare channels described by Zweifach and Metz (11) are either carrying a very small fraction of the total blood flow (few percent) or the blood within these channels is equilibrated with the tissue.

In-Parallel Compartments.—In the previous study (1) the washout curves were analyzed by a two compartment-in-parallel model. It was observed that after half an hour there remained a relatively large concentration of 133xenon in the slow compartment, while the fast compartment contained only about 10−4 of the former. If the local labelling experiments are considered, there should be such a concentration difference also within a tiny labelled fraction of the tissue. In the gas diffusion study previously mentioned (unpublished) it was found that such gradients were eliminated by diffusion within a few minutes. In addition, one should assume that the depot did not progress by diffusion into the muscle from the original site. If this occurred, the 133xenon of the slow compartment should only diffuse into a similar, but more deeply situated site having equally poor clearance conditions. The 133xenon of the fast compartment should only be able to diffuse into a deeper situated site with fast clearance conditions. Such an “in-parallel diffusion” is not considered probable. However, progression of the depot was in fact observed in the above mentioned unpublished study.

It is concluded that fixed anatomical, in-parallel compartments do not exist in skeletal muscle tissue.

In-Series Arrangement

Kety did not consider the possibility that the tracer could freely exchange between neighboring tissue cylinders and between arterial and venous vessels. This hypothesis of an in-series and mutually communicating system allowing redistribution or counter current exchange of 133xenon can explain the shape of the desaturation curve from skeletal muscle. This process, shunting by diffusion, can also explain the striking shape of the washout curve in skeletal muscle after an intra-arterial bolus injection of 133xenon in saline (1). In this previous study the initial clearance rate of the washout curve calculated from its first almost monoeXponential segment was faster (mean value 87% faster) than the directly measured blood flow. The clearance-rate then gradually decreased until a steady value was registered (the tail of the curve). This final clearance rate was slower than the directly measured blood flow (between 10 and 70% slower).

The hypothesis of counter current exchange in the resting gracilis muscle of dogs was recently put forward by Aukland et al. (12), who examined the hydrogen washout curve by a polarographic technique after saturation of the tissue. Aukland et al. tried to evaluate the size of the counter current exchange by applying a two compartment-in-parallel model to the venous concentration curves. The fast compartment was taken to indicate the shunt fraction. Aukland’s model involves that both compartments have homogeneous clearance conditions and well defined volumes of distribution without intercompartmental exchange. However, it appears most likely that there is a gradual increase from the greater arteries to the arterial end of the capillaries in the number of gas molecules, which are able to diffuse from the arterial to the venous blood. This implies a nonhomogeneous...
INERT GAS METHOD FOR MEASUREMENT OF BLOOD FLOW

Linear plot of a washout curve recorded after bolus injection (taken from reference (1) experiment no. 7). The monoexponential curve is constructed to have the same height and area and thus the same mean transit time as the recorded curve. The particles \( A_q \) have the transit time \( t_i \) in the recorded curve. If the washout curve was monoexponential then the transit time of these particles would be \( t_i \).

Clearance condition for the "shunt compartment." Auckland's model is therefore considered to be an oversimplification of the description of the shunt fraction. We suggest that a better description of the net size of the shunt is obtained by comparing the observed curve after an intra-arterial bolus injection with a hypothetical single exponential washout curve, both having the same mean transit time, i.e., the same heights and the same total areas under the curves. This is illustrated in Figure 7. It is seen from this figure that the tracer particles \( A_q \) were washed out \( t_i - t_f \) minutes faster than expected from the curve which would have been found if diffusion equilibrium between tissue and blood had been maintained throughout and the blood flow was uniformly distributed. The area between the two curves from zero time to their intersection at time \( t_i \) is interpreted as the net arterio-venous diffusion shunt. The area between the two curves from \( t_i \) to infinity, which has the same size, is taken to express the net size of the veno-arterial diffusion shunt.

This description of the size of the diffusion shunt is based on a washout curve after bolus injection. Obviously the same curves could be obtained by differentiation of desaturation curves after equilibration of the tissue by intra-arterial step input.

Exchange of highly diffusible inert gases through the walls of vessels greater than capillaries has also been demonstrated for krypton-85 in the skin in man (13). Stainsby and Otis (14) measured the oxygen uptake in skeletal muscle during exercise. They found that both by lowering the perfusion pressure (i.e., blood flow) and the arterial oxygen saturation the venous oxygen saturation decreased. However, at a venous oxygen saturation of about 10% the oxygen uptake of the muscle dropped rapidly. This indicates insufficient oxygen supply to the mitochondria of...
the muscle cell. As the function of the mitochondria in vitro has been found to be undisturbed at even lower oxygen tensions, then the results of the study can be interpreted as the oxygen was not offered to the mitochondria, i.e. the oxygen was shunted from the arteries to the veins.

Thompson et al. (15) compared the theoretically derived curve of D$_2$O uptake in skeletal muscle based on the assumption of flow limited uptake and homogeneity of blood flow with the experimentally observed venous outflow concentration curve. The observed discrepancy between the theory and the experimental results in their study can also be explained by the hypothesis of shunting of the highly diffusible D$_2$O molecules. This counter current exchange must be far more pronounced in heat clearance experiments as demonstrated in heart muscle tissue (16).

Perl et al. (8) and Rackow et al. (17) made an analysis for the compatibility of the perfusion-limited multicompartmental model of inert gas uptake in the whole body. The experimental data could not be accounted for by the model. Perl et al. explained the discrepancy as caused by bulk diffusion between the compartments. The finding in the present study that the shunting by diffusion invalidates the application of a single exponential uptake curve of inert gases must be taken into consideration in such multi-compartmental models for whole body uptake.

In the present study existence of shunting by diffusion from venous to the arterial blood could be assumed to disturb the equilibrium between tissue and venous blood and thus decrease the clearance rate. However, the venous blood will initially be kept almost equilibrated with the tissue even if it loses some amount of tracer to the arterial blood because the exchange area between tissue and venous blood is much larger than between arterial and venous blood. Therefore, the shunting by diffusion which can explain the shape of the curves does not necessarily invalidate the agreement between the directly-measured venous outflow and the blood flow calculated from the initial monoeponential slope of curves from an intra-arterially labelled muscle or from an atraumatically labelled local area.

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