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J. Robert Hippensteele
(Ph. D. Thesis)
August 29, 1967

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TRACER KINETIC STUDIES OF THE DYNAMICS OF THE MICROCIRCULATION IN PERIPHERAL VASCULAR BEDS

ABSTRACT

J. Robert Hippensteele

A theory that utilizes tracer kinetics has been developed and applied to the quantitative computation of relative changes in the extracellular mixing volume for an exchangeable substance injected into the arterial blood supply of a peripheral vascular bed. This mixing volume has been shown to increase by as large a factor as 2.9 in the hind limb of the dog during an adrenalin infusion. The experiments performed involved an intra-arterial injection of a mixture of an exchangeable and a nonexchangeable labeled substance and subsequent monitoring—with external energy-discriminating radiation detectors—of the tissue supplied by the artery, and simultaneous sampling (and subsequent analysis for the injected substances) of the venous blood leaving the tissue.

The spatially inhomogenous nature of the blood perfusion (i.e. the existence of different perfusion rates in different regions) of skeletal muscle tissue has been confirmed in these studies. That a large fraction of resting skeletal muscle tissue is not nutritively perfused is shown by the large increase in the extravascular mixing volume which becomes available to certain tracer substances during an adrenalin infusion. This suggests that a feedback mechanism controls the proportionate time spent in stasis and in flow by the blood in capillaries, thereby regulating the number of capillaries that are open at any instant in time.
Changes in the ratio of the injected substances in early samples of venous blood from the hind limb of the dog support the proposition that effective shunts exist in skeletal muscle vascular beds. Data are presented which indicate that this effective shunting of blood through the vascular bed could be, to some extent, a result of partial diffusion limitation on the clearance of the exchangeable substance from the plasma. All the data reported in these experiments are consistent with the concept that the effective shunts could include both anatomical shunts and such shunting mechanisms as partial diffusion limitation of the clearance of the test substance injected.
INTRODUCTION

A very important aspect of the general circulation in a mammalian system is the flow of blood through the minute vessels, e.g. the capillaries, in the peripheral vascular beds. It is at this level of the circulation, often called the microcirculation, that one of the major functions of the blood is carried out: the exchange of nutrients and metabolites between the blood and the extravascular fluids. Studies of the dynamics of the microcirculation are of obvious academic interest but they can also lead us toward advancements in the field of clinical medicine.

There are two basic approaches to the study of blood flow dynamics within a vascular bed. One approach is to obtain data while physically controlling certain parameters such as the blood pressure, the blood flow rate, and the affluent and effluent pathways involved in the vascular bed. In this approach, extirpated or isolated tissue is perfused with blood at constant flow rates or at constant perfusion pressures. The system being studied is therefore well defined and the resulting data are both precise and useful, but the conclusions drawn from such data may not apply to the vascular bed under physiological conditions.

The alternative approach is to obtain data without disturbing the physiological conditions of the vascular bed. Caution must be exercised to keep the experimental procedure simple but still obtain meaningful data. The analysis of the data must preclude the effects of the various uncontrolled parameters on the conclusions drawn from the data. For example, since the blood flow rate is under physiological control, the theoretical
analysis of the data obtained during the procedure must be independent of the blood flow rate or it must provide for the computation of relative changes in the blood flow rate and of the effect of these changes on the information obtained from the data.

In this thesis, a theory is presented which applies indicator dilution techniques to the study of changes in the distribution of blood flow which occur within a vascular bed, in skeletal muscle tissue, during physiological manipulations such as exercise, hypovolemic shock, and temperature change. The experimental technique required by the theory can be applied to trained, unanesthetized animals maintained in a physiological state. The theory is used in this thesis to analyze data obtained from anesthetized animals in which vascular changes were induced with an intravenous adrenalin infusion.

It has long been known that an adrenalin infusion produces an increase in muscle blood flow accompanied by a decrease in vascular resistance which indicates that vasodilatation occurs (8). More recently, Dobson and Warner (16) have shown that an intramuscular injection of adrenalin produces a change in the shape of the sodium-24 washout curve in muscle. They concluded that the nature of this change in shape indicates that the increased muscle perfusion rate could not be caused by a simple increase in flow rate through a static capillary bed, but could be caused by a nonuniform change in the character of the vascular network. All of these changes with adrenalin suggest that adrenalin administration could cause either the opening of previously closed capillaries or the nonuniform dilatation of open capillaries or both of these responses.

Let us assume that closed capillaries exist in skeletal muscle. The
extravascular tissue immediately surrounding a closed capillary is probably either poorly perfused or nonperfused. The opening of capillaries in a previously nonperfused volume of the tissue would cause an increase in the volume of skeletal muscle tissue being perfused. Conversely, an increase in the tissue perfusion volume would indicate that some capillaries in previously nonperfused tissue have become open. An increase in the perfusion volume accompanied by an increase in the perfusion rate (the blood flow rate per unit of perfusion volume) would indicate that the blood flow rate increased by a larger factor than the perfusion volume. That these hypothetical changes actually occur in skeletal muscle during an adrenalin infusion is demonstrated in this thesis. Such changes suggest that, in addition to the opening of capillaries in previously nonperfused tissue, adrenalin causes an increase in the diameters of previously open capillaries or an increase in the number of capillaries in a region of the tissue which was already being perfused.

This redistribution of the capillary blood flow could be further complicated by the existence of arteriovenous anastomoses able to open and close. Some investigators have suggested the existence of nonnutritional blood flow in skeletal muscle (5,7,9,19,24,38,52) which could involve such anastomoses. A more complete description of the redistribution of the capillary blood flow should be possible if we simultaneously determine the directional changes in the nonnutritional blood flow rate and the directional changes in the perfused volume.

The theory presented in this thesis utilizes tracer kinetic studies of two labeled, intra-arterially injected substances to simultaneously
measure changes in the perfused volume and the nonnutritional blood flow. These tracer kinetic studies are performed on experimental animals before and during an adrenalin infusion to determine the effect of adrenalin on the parameters studied. The results suggest that, although adrenalin causes an increase in the number of open capillaries and in the diameters of the capillaries, it also causes a decrease in the fractional nonnutritional blood flow.
THEORY OF RELATIVE VOLUMES

The model to be considered consists of skeletal muscle tissue surrounding the small vessels which connect a single artery to a single vein. Blood flows from the artery, through the small vessels to the vein. In the model, I will designate some of the small vessels as capillaries which allow certain substances, subsequently called exchangeable substances, to exchange between the intravascular and the extravascular fluids. I will designate the remainder of the small vessels as shunts which allow little or no exchange to occur across the vascular wall. The anatomical relationship between the various small vessels in this model are not important to the work presented in this paper but the presence and functional responses of these vessels are important. The essential elements of the model, demonstrated in Fig. 1, combine the functional, but not the complex anatomical, aspects of the various descriptions of the skeletal muscle vascular bed which have been presented by several authors (1,2,12,28,30,40,44,52,53).

The amount of time required for a small particle introduced into the artery to appear in the vein depends on its path through the system. This means that there is a distribution of transit times for a substance flowing through the system. Meier and Zierler (31) have shown that, for such a system, the mean transit time, \( \bar{t} \), for a number of particles introduced into the artery is always equal to the exchange volume, \( V \), available to those particles divided by the volume flow rate, \( F \), of the intravascular fluid carrying the particles. That is,
Fig. 1. A schematic representation of the essential microcirculatory elements of a peripheral vascular bed. This diagram does not represent the true anatomy of such beds.
Following an intra-arterial injection into the system, a substance will be distributed by the blood flow to the spaces available to that substance. Subsequent blood flow will wash the substance out of these spaces. The time function of the amount of the substance present in the system will be represented by \( q(t) \) and the time function of the concentration of the substance in the venous blood leaving the system will be represented by \( c(t) \). Meier and Zierler (31) have shown that, following an intramuscular injection,

\[
\int_{0}^{\infty} q(t) \, dt = q_o \bar{t},
\]

where \( q_o \) is the amount of substance injected at time zero.

Equation (2) also applies to an intra-arterial injection of the substance if mixing is essentially instantaneous, that is, if the substance equilibrates across all of the available capillary walls of the system before an appreciable amount of the substance has left. The advantage of the intra-arterial injection over the intramuscular injection has been discussed by Dobson and Warner (16,45).

Substitution of \( \bar{t} \) from Eq. (1) into Eq. (2) gives us

\[
\int_{0}^{\infty} q(t) \, dt = q_o \frac{V}{F} \]

or

\[
\frac{V}{F} = \frac{\int_{0}^{\infty} q(t) \, dt}{q_o}.
\]
In the model being considered, all the injected substance, $q_0$, must flow past the venous sampling site. Since the amount of substance which flows past the sampling site during the time interval, $dt$, is the product of the volume flow past the site $F \cdot dt$, times the concentration at the site at that time, $c(t)$,

$$q_0 = \int_0^\infty Fc(t) \, dt.$$  

If it is assumed that the flow is constant, this equation can be solved for $F$, giving us

$$F = \frac{q_0}{\int_0^\infty c(t) \, dt}. \quad (4)$$

The mathematical treatment of the model to this point has been described in more detail by Zierler (48).

Substituting $F$ from Eq. (4) into Eq. (3) and solving for $V$ gives a theoretically simple determination of the mixing volume available to the injected substance. However, in practical experiments, it is difficult to monitor externally with constant counting efficiency all (but only) the tissue fed by a single artery and drained by a single vein. It is also difficult to correct accurately for the difference in the counting efficiency of the tissue detector and of the blood sample detector. These difficulties can be avoided by determining relative, rather than absolute, mixing volumes. The former are adequate for the present analysis.

Suppose two substances, one exchangeable and one nonexchangeable, are simultaneously injected into the artery, as described by Chinard,
Vosburgh, and Enns' (11) and by Anthonisen and Crone (4). If neither substance enters the red blood cells, the flow rates carrying each substance through the system are equal.

Then
\[
\frac{V_{\text{exch}}}{V_{\text{nonexch}}} = \frac{F_{\text{exch}}}{F_{\text{nonexch}}},
\]
(5)

where the subscripts denote whether the values refer to the exchangeable substance or to the nonexchangeable substance.

The numerator and the denominator of the left-hand side of Eq. (5) can each be replaced by the appropriate solution of Eq. (3). Therefore,
\[
\frac{q_0}{q_{\text{exch}}} \int_0^\infty q_{\text{exch}}(t) \, dt = \frac{V_{\text{exch}}}{V_{\text{nonexch}}},
\]
(6)

Since the nonexchangeable substance does not enter the red blood cells, the plasma volume,
\[
V_{\text{pl}} = V_{\text{nonexch}}.
\]
(7)
The value of \( V_{\text{exch}} \) represents a virtual volume, the volume in which the exchangeable substance would be mixed if the extravascular concentration were equal to the intravascular concentration. The actual volume of extravascular tissue, \( V_{\text{tm}} \) in which the exchangeable substance mixes is, therefore,
\[
V_{\text{tm}} = k(V_{\text{exch}} - V_{\text{nonexch}}),
\]
(8)
where \( k \) is a steady-state distribution coefficient. That is
Let $\rho$ be the ratio of the extravascular tissue mixing volume to the plasma volume. Then $\rho$ is equal to Eq. (8) divided by Eq. (7),

$$
\rho = \frac{k(V_{\text{exch}} - V_{\text{nonexch}})}{V_{\text{nonexch}}};
$$

or,

$$
\rho = k \left( \frac{V_{\text{exch}}}{V_{\text{nonexch}}} \right) - k.
$$

Now $\rho$ can be evaluated in terms of the integrals of Eq. (6).

$$
\rho = k \left( \frac{q_{\text{noexch}}^{\infty} \int_{0}^{\infty} q_{\text{exch}}(t) \, dt}{q_{\text{exch}}^{\infty} \int_{0}^{\infty} q_{\text{nonexch}}(t) \, dt} \right) - k. \quad (9)
$$

The effect of adrenalin infusion on the value of $\rho$ can be computed as the ratio

$$
A = \frac{\rho_{\text{adren}}}{\rho_{\text{control}}},
$$

where the superscripts denote whether the data were obtained during (adren) or before (control) the adrenalin infusion. The value of $A$ will indicate whether adrenalin increases or decreases the tissue-mixing volume relative to the plasma volume. For example, a value of $A$ greater than unity
indicates an increase in the mixing volume relative to the plasma volume. This information does not show whether the respective volumes actually increased or decreased. The directional changes in the volumes can be evaluated as described below.

Equation (3) states that

\[ \frac{v_{\text{adren\ nonexch}}}{v_{\text{adren}}} = \frac{\int_0^\infty q_{\text{nonexch}}(t) \, dt}{\int_0^\infty q_{\text{nonexch}}(t) \, dt}, \]

and a similar relationship holds for the control conditions. Then, since

\[ v_{\text{pl\ nonexch}} = v_{\text{nonexch}}, \]

\[ v_{\text{pl\ control\ control}} = \frac{\int_0^\infty q_{\text{nonexch}}(t) \, dt}{\int_0^\infty q_{\text{nonexch}}(t) \, dt} \cdot \]

Let \( R_{\text{pl}} \) be the ratio of the plasma volume during an adrenalin infusion to the plasma volume before the infusion. By rearrangement of the above equation, we get

\[ R_{\text{pl}} = \frac{\frac{v_{\text{pl}}}{v_{\text{pl}}}}{\frac{v_{\text{pl}}}{v_{\text{pl}}}} = \left( \frac{\int_0^\infty q_{\text{control}}(t) \, dt}{\int_0^\infty q_{\text{control}}(t) \, dt} \right) \left( \frac{\int_0^\infty q_{\text{nonexch}}(t) \, dt}{\int_0^\infty q_{\text{nonexch}}(t) \, dt} \right). \] (10)

The numerator and the denominator of the second term in parentheses in Eq. (10) can each be replaced by the appropriate solution of Eq. (4). Then

\[ R_{\text{pl}} = \frac{\int_0^\infty q_{\text{control}}(t) \, dt}{\int_0^\infty q_{\text{control}}(t) \, dt} \left( \frac{\int_0^\infty q_{\text{adren}}(t) \, dt}{\int_0^\infty q_{\text{control}}(t) \, dt} \right). \] (11)
Let $R_{tm}$ be the ratio of the extravascular tissue volume available for mixing of the test substance during an adrenalin infusion to that volume before the infusion. Then $R_{tm}$ is the product of $R_{pl}$, the ratio of the respective plasma volumes, times $A$, the ratio of the respective values of $p$. That is,

$$R_{tm} = \frac{v_{adren}}{v_{control}} = R_{pl} \cdot A.$$  

(12)
THEORY OF EFFECTIVE SHUNTS

After the simultaneous injection of an exchangeable and a nonexchangeable substance into an artery a qualitative analysis of the function $R_c(t)$, where $R_c$ is the ratio of the concentration of exchangeable test substance to the concentration of nonexchangeable reference substance in the venous blood, can indicate whether or not the system contains effective shunts.

In this discussion, an effective shunt is any structure or mechanism which allows blood to travel from an artery to a vein without complete equilibration of the plasma sodium with the tissue sodium. If no shunts exist in the system, the first activity appearing in the venous blood should reflect maximal loss of the exchangeable substance relative to the nonexchangeable substance. This loss would decrease with time, causing $R_c$ to increase with time. Eventually $R_c$ would become greater than 1.0, since the nonexchangeable substance washes out of the system faster than the exchangeable substance. The expected shape of $R_c(t)$ for such a system is shown in Fig. 2A.

For this model, the distribution of transit times has been assumed to be a normal or skewed normal distribution.

If the system contains only shunts, the exchangeable substance would not leave the intravascular fluid. Hence, $R_c(t)$ is shown in Fig. 2B to be constant at 1.0 for such a system.

If the system contains both capillaries and shunts, and if the transit times through the capillaries tend to be longer than the transit times through the shunts, the early venous blood samples would contain some blood which had passed through shunts and perhaps some blood which had passed
Fig. 2. The theoretical variations of the function $R_c(t)$ in the venous blood of three functionally different vascular beds.
through capillaries. The value of $R_c$ for each sample would depend on the relative amounts of shunted and capillary blood in the sample. The result would be a flattening of, or possibly a dip to a minimum in, the early portion of the curve, $R_c(t)$. The earliest venous blood sample could conceivably contain only shunted blood, in which case $R_c$ would equal unity, but subsequent samples would contain increasingly less shunted and more capillary blood, causing the curve $R_c(t)$ to approach the theoretical curve in Fig. 2A. The possible shapes of $R_c(t)$ at early times in this system are shown by broken lines in Fig. 2C.

It must be pointed out that, if $R_c(t)$ for an experimental system has a shape similar to the curve in Fig. 2C, an effective shunt must exist for the test substance used, but this result does not prove that an anatomical shunt exists. For example, if the blood flow through the capillaries were so rapid that essentially complete equilibration of the exchangeable substance between the intravascular and the extravascular fluids could not occur, the $R_c(t)$ curve would have a shape similar to the curve in Fig. 2C. The early samples would contain more blood which had not equilibrated than later samples, resulting in an early flattening or dipping to a minimum in the $R_c(t)$ curve. Such diffusion limitation of the exchange of a given substance can be considered to be a "shunting mechanism" for that substance.
Methods

Female beagle dogs, weighing 9 to 11 kilograms, were anesthetized with sodium pentobarbital (30 mg per kg body weight). With the dog in a supine position, both hind legs were extended so that part of the biceps femoris and the underlying muscles of each leg were in the detection field of separate energy-discriminating radiation detectors. Rapid data collection of up to ten data points per second was made possible by storing the data from the discriminators and the time of day in a PDP-8 computer (Digital Equipment Corporation) until the data could be punched on paper tape. This data acquisition is described under the heading "Data Acquisition with In-Vivo Detectors" in the next section of this thesis. Venous blood samples were taken rapidly at about one sample per second. Polyethylene tubing was inserted into the dorsal branch of the lateral saphenous vein or into the medial saphenous vein of each hind leg and threaded proximally to the femoral vein in the inguinal region. Absorbent paper, taped to a motor-driven drum, collected drops of blood from the distal ends of these tubes. Coagulation of the blood within the tubing was prevented by an intravenous injection of 1.0 ml of sodium heparin solution into the dog fifteen minutes before the start of the experiment.

A small volume (0.14 to 0.20 ml) of a solution containing an exchangeable substance, 30 μCi of ionic sodium-24, and a nonexchangeable substance, 0.2 mCi of colloidal gold-198, made isotonic with NaCl, was rapidly injected into the right femoral artery in the inguinal region. The injection was made through tubing connected to an indwelling arterial needle.
which remained in place throughout the experiment. Blood flow to both paws was occluded by applying supra-arterial pressure to pneumatic cuffs around each hind limb proximal to the paw. This pressure was released after the last blood sample was collected, approximately 3 minutes after the injection.

The data from the detector below the right leg gave sodium and colloidal gold disappearance curves. The data from the detector below the left, noninjected, leg gave appearance curves for the two substances carried to that leg by the circulation. Let us assume that the blood flow to both legs is the same. Then, to correct for recirculation, we can subtract the appearance curve from the disappearance curve for each substance. The resulting curve, \( q(t) \), is the net disappearance curve for that substance.

The blood samples (drops) were analyzed for sodium-24 and gold-198 by utilizing the difference in their decay rates. A 5-day interval elapsed between two countings of each sample. Then solutions were obtained for two simultaneous equations which describe the activity observed at each counting of a single sample. This analysis of the blood samples is described in a later section of this thesis under the heading "Data Acquisition from the Blood Samples."

The average size of the blood samples (drops) was found to be about 0.02 ml. Assuming that the drop sizes were essentially constant, the venous blood concentration of the labeled substances was obtained in units of counts per minute (CPM) per drop which are equivalent to units of CPM per 0.02 ml. Curves of the venous blood concentration of each substance in the injected and the noninjected leg were constructed.
These curves were very smooth and therefore supported the assumption of a
constant drop size. Again we will assume that the blood flow to both
legs is the same. The curve for one substance in the noninjected leg is
subtracted from the curve for that substance in the injected leg to cor-
rect for recirculation. The resulting curve is $c(t)$, the concentration of
the substance in the venous blood of the injected leg resulting from the
washout of that substance from the tissues of that leg.

When the net disappearance curve, $q(t)$, for sodium had reached a low
value (less than 1% of its peak value), adrenalin was infused into the
jugular vein at a rate of 0.21 to 3.03 μg/min per kilogram body weight.
The response of the dog to the adrenalin was monitored by a continual
recording of the pulse pressure, which increased after the adrenalin
infusion was begun. When the pulse pressure reached a constant value, a
second injection of the labeled substances was given. The geometry of
the devices for data and blood-sample collection remained unchanged for
the two parts of each experiment.

In two experiments, the injectate consisted of 50 μCi of $^{14}\text{C}$
sucrose and 30 μCi of $^{24}\text{NaCl}$ in 0.25 ml of an isotonic solution. Since
the in-vivo detectors could not detect the low-energy beta particles
from $^{14}\text{C}$, the time function, $q(t)$, of the amount of each isotope present
in the leg was not recorded. The analysis of the blood samples was per-
formed in the same way as described for the $^{24}\text{Na}/^{198}\text{Au}$ samples.

One of the $^{14}\text{C}$ experiments included an adrenalin infusion as de-
scribed above. During the other $^{14}\text{C}$ experiment, the adrenalin
infusion was not performed. Instead, after the control part of the experi-
ment was completed, a second injection was given while the muscles of the
injected hind limb were being stimulated. Two platinum-needle electrodes from a Grass stimulator were inserted, one into the gracilis muscle near its distal end, the other into the semitendinosus muscle at its midpoint. The stimuli were given at a rate of 5 per sec with a duration of 1.5 sec and a strength of 12 volts.
DATA ACQUISITION WITH IN-VIVO DETECTORS

In order to monitor the amount of injected sodium-24 and gold-198 present in the muscles of each hind leg of the dog, separate 2 x 2-in. NaI(Tl) crystal radiation detectors were mounted below two 1-in. diameter holes in a piece of lead 1.5 by 6 by 11 in. The hind legs of the dog were positioned so that the holes acted as collimators for photons from the decaying isotopes in part of the biceps femoris and the underlying muscles of each leg. The crystal detectors were bonded to photomultiplier (PM) tubes and were shielded on all sides by 2 in. of lead.

Pulses from each PM tube were amplified and fed into a pair of single-channel pulse-height analyzers. One of the analyzers of each pair was adjusted to pass the peak intensity pulses resulting from sodium-24 decay during which both a 2.76-MeV and a 1.38-MeV photon are emitted (43.) The other member of each pair of analyzers was adjusted to pass the pulses resulting from gold-198 decay, during which a 0.41-MeV photon is most often emitted (43.) Narrowing the window of such an analyzer in the region of the energy of a characteristic peak intensity photon increases the analyzer's capability for discriminating between isotopes but decreases the counting efficiency of the system for the isotope of interest. The best window adjustment for an analyzer which is to detect a given isotope is found by gradually narrowing the window until, in effect, only that isotope is detected or until the lowest tolerable counting efficiency for that isotope is reached. The lowest tolerable counting efficiency is determined by considering the amount of the isotope which will be present
and the number of counts required at each data point to give statistically meaningful data.

Radioactive decay can be approximated by a Poisson distribution. For a Poisson distribution, the standard deviation (21) is

\[ \sigma = (npq)^{1/2}, \]

where \( n \) is the number of atoms present, \( p \) is the probability that an atom will decay during the time \( t \), and \( q \) is \((1.0 - p)\). Since \( p \) is very small for sodium-24 and gold-198, \( q \) will be approximately 1.0. The product \((np)\) is the average total counts, \( N \), observed during the time interval, \( t \). Therefore, the standard deviation for \( N \) counts observed during the time interval, \( t \), can be approximated by the value \( N^{1/2} \). This means that in 68% of a large number of measurements, each lasting for a length of time \( t \), the number of counts observed would be within the range \( N \pm N^{1/2} \). By requiring that \( \sigma \) never exceed 0.05\( N \), a minimum value of \( N \) for any data point was set at 400 counts. A data collection rate of ten samples per second would therefore require that \( 2.4 \times 10^5 \) cpm be detected. In order to allow the injected volume of labeled substances to be small (about 0.2 ml), the total counting efficiency of the detectors had to be at least 0.4% for sodium-24 and 0.05% for gold-198 distributed in the tissue of the leg. This set the limit on the degree to which the windows of the analyzer could be narrowed.

Although photons from a given isotope have discrete energies, the interactions of a large number of such photons with tissue, air, and the material of the detectors result in a continuous energy spectrum of
photons in the detector. This effect is caused by Compton scattering, which accounts for most of the energy loss of the photons from sodium-24 and gold-198 (18). The resulting continuous energy spectrum has characteristic peaks which depend on the characteristic photon energies and on the size, shape, and type of detector used. Because of the continuous photon energy spectrum, it is impossible to eliminate completely from a given analyzer the passage of pulses resulting from the decay of the unwanted isotope. This appearance of pulses from the "wrong" isotope will be called "window crosstalk."

The crystal detectors were well shielded but, due to the anatomy of the dog, they had to be situated very close together, causing their sensitive fields to overlap. Therefore, photons from the isotopes in one leg could be detected by the detector under the opposite leg. This phenomenon will be called "detector crosstalk."

The pulses from each analyzer plus the pulses from an electronic clock were fed into an input buffer of a PDP-8 computer and were subsequently entered, via a TAD (two's complement add) instruction, into separate locations assigned to each analyzer and the clock. Subsequent pulses from the analyzers and clock were TADed into their assigned locations until a predetermined number of counts or a predetermined number of clock ticks had been accumulated in any of the five locations. When this occurred, the resulting binary words in the five locations were transferred to another section of memory to be stored until they could be punched on paper tape. The computer was programmed to use one set of limits for the maximum number of counts and the maximum elapsed time when the
counting rate exceeded a predetermined level, and another set of limits when the counting rate fell below that level. Thus a high rate of data acquisition of up to ten data points per second occurred when the counting rate from any of the four analyzers exceeded $3 \times 10^4$ cpm. When this criterion was not met, the rate of data acquisition decreased with the counting rate, reaching a minimum value of about two data points per minute when the counting rates at all four analyzers were less than $8 \times 10^3$ cpm. During the injection of the isotopes, the amount of activity present in the tissues of the injected leg increased rapidly to an early peak and then decreased rapidly during the first several seconds after the injection. Rapid data acquisition was necessary during this early portion of the curve. Continued rapid data sampling was not necessary; nor was it possible, since the paper tape punch is relatively slow (10 characters per second) and the PDP-8 computer memory has only 4000 word locations. The memory would soon overflow with the rapid rate of data input and the relatively slow rate of data output.

The paper tape output from the PDP-8 computer contains the data in the form of a series of five logical binary words. In each set of five words, the first word represents the elapsed time and the other four represent the accumulated counts in each of the four input channels. The paper tape was read into a DDP-24 computer (Computer Control) which converted the PDP-8 12-bit word format to a 60-bit word format for compatibility with the CDC 6600 computer. Then a magnetic tape containing the converted data from the paper tape was generated for further analysis on the CDC computer.
For each data point, the counts accumulated in each of the four input data channels were divided by the number of clock ticks and converted to cpm. Then each data channel contained the activity of one isotope in one leg plus the activity resulting from window crosstalk from the other isotope in that leg and from detector crosstalk from both isotopes in the opposite leg. For each data point, therefore, a system of four equations (one for each channel) and sixteen unknowns (four for each channel) could be used.

Calibration procedures made it possible to evaluate the twelve unknowns which resulted from crosstalk in terms of the other four unknowns, giving a system of four equations and four unknowns. In order to describe the calibration procedures, I will designate as detector #1, the detector under the leg into which the isotopes were injected, and as detector #2, the detector under the opposite leg. For each detector, window a will represent the analyzer which was adjusted for isotope A, sodium-24, and window b will represent the analyzer which was adjusted for isotope B, gold-198. Therefore, the data in channel 1-a will be the activity which was detected through window a of detector #1. The other three data channels are 1-b, 2-a, and 2-b.

Since the photons from the $^{24}\text{Na}$ are of different energies than those from the $^{198}\text{Au}$, the intensity loss per unit of absorber (tissue) thickness is not equal for the two sources of photons(18). Therefore, the counting geometry used for the calibration procedures must simulate the counting geometry which existed during the experimental procedure. The mass absorption coefficients for photons with energies between 0.1 and 3.0 MeV
are essentially equal, at a given energy, for water (a good approximation for muscle tissue) and for fat tissue, and are not too different for bone tissue (3). Since the specific gravity of soft tissue is approximately the same as that of water, the latter was used as a tissue-equivalent absorber in the calibration procedures for these experiments.

$^{24}$Na and $^{198}$Au were homogeneously distributed in water in plastic containers with a counting geometry similar to the geometry of the muscle viewed during the experiments. The solution of $^{24}$NaCl was placed over counter #1 and the counting rates at all four windows were obtained. These counting rates will be represented as $X_{1-a}$, $X_{1-b}$, $X_{2-a}$, and $X_{2-b}$, where the subscripts represent the channel in which the data appear as described earlier. The window crosstalk factor for $^{24}$Na at detector #1 was

$$a_1 = \frac{X_{1-b}}{X_{1-a}}$$

and the detector crosstalk factor for $^{24}$Na at detector #1 was

$$\alpha_1 = \frac{X_{2-a}}{X_{1-a}}$$

The $^{24}$NaCl was transferred to detector #2 and new data were recorded. Then the window crosstalk factor for $^{24}$Na at detector #2 was

$$a_2 = \frac{X_{2-b}}{X_{2a}}$$

and the detector crosstalk factor for $^{24}$Na at detector #2 was
Similarly, a suspension of colloidal $^{198}$Au was used to determine the window crosstalk factors, $b_1$ and $b_2$, and the detector crosstalk factors, $\beta_1$ and $\beta_2$, for $^{198}$Au. The values obtained for these crosstalk factors in one experiment (\#166) are as follows.

\[
a_1 = 0.063 \quad \alpha_1 = 0.031
\]
\[
a_2 = 0.083 \quad \alpha_2 = 0.028
\]
\[
b_1 = 0.0014 \quad \beta_1 = 0.000
\]
\[
b_2 = 0.0021 \quad \beta_2 = 0.000
\]

These values were quite constant from one experiment to the next.

When both $^{24}$Na and $^{198}$Au are present above both detectors, the eight crosstalk factors can be entered as coefficients into the system of four equations describing the data which appear in the four channels. These equations can be written as follows:

\[
X_{1-a} = A_{1-a} + \alpha_2 A_{2-a} + b_1 B_{1-b} + b_2 B_{2-b},
\]
\[
X_{1-b} = a_1 A_{1-a} + a_1 \alpha_2 A_{2-a} + B_{1-b} + \beta_2 B_{2-b},
\]
\[
X_{2-a} = \alpha_1 A_{1-a} + A_{2-a} + b_2 \beta_1 B_{1-b} + b_2 B_{2-b},
\]
\[
X_{2-b} = a_2 \alpha_1 A_{1-a} + a_2 A_{2-a} + \beta_1 B_{1-b} + B_{2-b},
\]

where $X$ is the total activity observed, $A$ is the activity of isotope $A$, $B$ is the activity of isotope $B$, and the subscripts represent the window
through which the counts were observed.

In order to solve this system of equations for the unknowns $A_{1-a}$, $A_{2-a}$, $B_{1-b}$, and $B_{2-b}$, it was written as the matrix equation,

$$
\begin{bmatrix}
1 & a_2 & b_1 \\
 a_1 & a_1a_2 & 1 \\
 a_1 & 1 & b_2b_1 \\
 a_2a_1 & a_2 & b_1
\end{bmatrix}
\begin{bmatrix}
A_{1-a} \\
A_{2-a} \\
B_{1-b} \\
B_{2-b}
\end{bmatrix}
= 
\begin{bmatrix}
X_{1-a} \\
X_{2-a} \\
X_{1-b} \\
X_{2-b}
\end{bmatrix}
$$

Then, if the coefficient matrix is nonsingular (41),

$$
\begin{bmatrix}
A_{1-a} \\
A_{2-a} \\
B_{1-b} \\
B_{2-b}
\end{bmatrix}
= 
\begin{bmatrix}
1 & a_2 & b_1 & b_1b_2 \\
 a_1 & a_1a_2 & 1 & b_2 \\
 1 & 1 & b_2b_1 & b_2 \\
 a_2a_1 & a_2 & b_1 & 1
\end{bmatrix}^{-1}
\begin{bmatrix}
X_{1-a} \\
X_{2-a} \\
X_{1-b} \\
X_{2-b}
\end{bmatrix}
$$

At the beginning of each experiment standards were counted as described above. The values obtained for the crosstalk factors were used to obtain the coefficient matrix, which was then inverted and subsequently used to solve the matrix equation for each data point. Since no geometry changes occurred during the experiment, it is valid to assume that the crosstalk factors remained constant during each experiment.

The isotopes were homogeneously distributed in the calibration standards but were not homogeneously distributed in the tissues after injection into the hind limb of the dog. We can assume that the cells contained essentially no isotopes (for discussion, see page 40), that the extra-
vascular-extracellular fluid contained only $^{24}\text{Na}$ and that the blood contained both $^{24}\text{Na}$ and $^{198}\text{Au}$. Most of the data analysis for these experiments involved determining the ratio of the amounts of the two isotopes present. In order to test the validity of using homogeneous distributions of the isotopes during the calibration procedures, the ratios of a given mixture of the two isotopes were computed by use of data obtained from a homogeneous and from a heterogeneous distribution of the isotope mixture. The crosstalk factors were determined as described above. Then, for the homogeneous distribution, a sufficient amount of a mixture of $^{24}\text{Na}$ chloride and colloidal $^{198}\text{Au}$ was added to some water in a plastic container over one of the detectors to give a counting rate of about $10^5$ cpm. The water level in the container was adjusted to about 8 cm. After mixing, the solution was counted over each detector to determine the ratio $^{24}\text{Na}/^{198}\text{Au}$. Into a similar plastic container of water, a flat piece of plastic was inserted so that the walls of the container held the plastic in an upright position. Portions of the original mixture of isotopes were injected into a length of polyethylene tubing which had been taped to the side and bottom edges of the piece of plastic until the counting rate at the detector below the container reached $10^5$ cpm. This heterogeneous distribution of the isotopes was counted with the horizontal (bottom) portion of the tubing at a distance of 1 cm from the bottom of the container. Counting was repeated with the tubing at distances of 2, 3, 4, 5, and 6 cm from the bottom of the container. At each distance, the ratio of $^{24}\text{Na}/^{198}\text{Au}$ was determined and then divided by the ratio obtained for the homogeneous distribution of the isotopes. At all the distances, the
resulting normalized ratio was essentially 1.0 for the heterogeneous distribution of the isotopes. Therefore, it is valid to use the homogeneous distributions of the isotopes for the calibration procedures.

In the methods section of this thesis, the correction for recirculation of the isotopes in the dog was described. In order to make this correction, the appearance curves obtained for each isotope at detector #2 were subtracted from the disappearance curves obtained for the isotopes at detector #1. For this procedure, it was necessary to account for the difference in the counting efficiencies of the two detector circuits. The counting efficiency in detector #2 relative to detector #1 was obtained for each isotope by dividing the counting rate from detector #2 when the isotope was counted at detector #2 during the calibration procedure by the counting rate from detector #1 when the isotope was counted at detector #1. All subsequent data from detector #2 were divided by the appropriate value for the relative counting efficiency.

Similar to the need for relative counting efficiency corrections was the need for relative counting geometry corrections. It would not be valid to assume that the two detectors viewed equal volumes of tissue, but, since the dog was not moved during the experiment, it is valid to assume that the relative counting geometry remained constant throughout each experiment.

When the slope of the gross disappearance curve (before being corrected for recirculation) for $^{24}\text{Na}$ becomes zero, the $^{24}\text{Na}$ is entering the injected leg at the same rate as it is leaving. At this point, the tissue concentration of $^{24}\text{Na}$ should be equal in the two hind limbs. When this condition exists, the $^{24}\text{Na}$ counting rate at each detector is proportional
to the volume of tissue viewed by that detector. A geometry factor can be defined as the volume of tissue viewed by detector #1 divided by the volume of tissue viewed by detector #2, which is equal to the $^{24}\text{Na}$ counting rate at detector #1 divided by the $^{24}\text{Na}$ counting rate at detector #2 when the slope of the gross $^{24}\text{Na}$ disappearance curve is zero. This zero slope always existed for the last 5-minute portion of the curve of the data obtained during the adrenalin infusion. Therefore, the geometry factor was computed from the data obtained during the last 5 minutes of the adrenalin infusion. Then, all data obtained from detector #2 during both parts of the experiment were multiplied by the geometry factor to correct for the relative volumes of tissue viewed by the two detectors.

The NaI(Tl) crystal detectors and the associated electronic circuits were designed for counting rates of $10^6$ cpm with very little loss in counting efficiency due to detector dead time. (The dead time is the length of time after detection of a photon that the detector, including the circuit, is not sensitive to subsequent photons.) Since counting rates as high as $3.5 \times 10^5$ cpm were encountered in these experiments, it was necessary to determine the actual dead times for each isotope at each detector. The dead time, $\tau$, of the detection apparatus is defined by the equation

$$R = \frac{N}{t - N\tau}, \quad (13)$$

where $R$ is the true counting rate and $N$ is the number of counts observed during a time interval, $t$. The total time that the detection apparatus
was not sensitive to photons during the time interval, \( t \), is \( N \tau \). The power series expansion of Eq. (13) is

\[
R = \frac{N}{\tau} \left( 1 - \frac{N \tau - 1}{t} \right) = \frac{N}{\tau} \left( 1 + \frac{N \tau}{t} + \frac{N^2 \tau^2}{2} + \cdots \right).
\]

If the value of \( \tau \) is sufficiently small, \( R \) can be approximated by the first two terms of the expansion. Then

\[
R \approx \frac{N}{t} + \left( \frac{N}{t} \right)^2 \tau.
\]

Equation (14)

The value of \( \tau \) was obtained by the paired-sample counting technique (27). Two samples of a single isotope which had approximately equal counting rates were used. Sample A was placed over the detector and counted to obtain \( R_a \). Sample B was placed over the detector next to sample A, without moving sample A, and both samples were counted to obtain \( R_c \). Then sample A was removed, without moving sample B, and sample B was counted to obtain \( R_b \). Using the approximation of Eq. (14) and using capitalized subscripts to represent the true counting rates gives the relationship

\[
R_c - b \approx R_c + (R_c)^2 \tau - b = R_a + R_b - 2b = R_a + (R_a)^2 \tau + R_b + (R_b)^2 \tau - 2b,
\]

where \( b \) is the background activity. Therefore,

\[
R_c + (R_c)^2 \tau \approx R_a + R_b + (R_a^2 + R_b^2) \tau - b,
\]

and
Values for each isotope at each detector were obtained for two sets of paired samples, one at the lower end and the other at the higher end of the range of counting rates encountered in the experiments. In all these determinations, the two values of \( \tau \) obtained for a single isotope at one detector were essentially equal. Therefore, the first two terms of the power series expansion of Eq. (13) are adequate for the detection equipment used. All the data obtained with this equipment were corrected for dead time by using the following values of \( \tau \) for \( ^{24} \text{Na} \),

\[
\tau_1 = 1.54 \times 10^{-7} \text{ min}, \quad \tau_2 = 2.51 \times 10^{7} \text{ min},
\]

and for \( ^{198} \text{Au} \),

\[
\tau_1 = 3.23 \times 10^{-7} \text{ min}, \quad \tau_2 = 3.75 \times 10^{-6} \text{ min}.
\]

In addition to the corrections which have been described, all the data were corrected for background activity and for the decay of each isotope from the time of calibration (the counting of the standards) to the time of data collection.
DATA ACQUISITION FROM THE BLOOD SAMPLES

Absorbent paper (Whatman No. 1) was cut into 0.75-in. strips and mounted on double-coated Scotch tape which had been placed along a spiral line around a variable-speed motor-driven drum. The drum was rotated on a screw which drove it laterally at a rate which kept the strip of absorbent paper under the stationary end of an indwelling venous blood sampling catheter. The drum was effectively divided in half, one half being used to collect samples from the injected leg, the other half being for the noninjected leg samples collected concurrently. The speed of the drum was adjusted to produce separate samples of the blood, which was dripping from the ends of the catheters. A marker syringe was connected to the injection syringe so that the plungers of both syringes moved in parallel. Polyethylene tubing (i.d. = 0.023 in.) from the marking syringe was connected to a hubless 22-ga needle which was mounted above the absorbent paper. The marking syringe and the tubing were filled with fluid, after which a few drops of ink were drawn into the needle and tubing. During the injection, a head of pressure produced in the marking syringe forced ink out of the needle and onto the paper, leaving a mark which represented the injection. Then, the time of sample collection for each blood sample was calculated as the distance between the sample and the injection marker divided by the velocity at which the paper strip passed the ends of the sampling catheters. The samples were covered with clear tape (except the samples containing $^{14}$C), labeled according to their distances from the injection marker, cut apart, and placed on copper disks to be counted on
a low-background gas-flow counter (Sharp Widebeta).

Analysis of the blood samples for the amounts of each isotope present was based on the difference in their decay rates and on the different β-particle energies emitted during the decay of the two isotopes. Each sample was counted twice. During the first counting, an absorber was placed between the sample and the detector. For the absorber, a material was chosen which would greatly decrease the detected amount of the longer-lived isotope relative to the shorter-lived isotope. No absorber was used during the second counting of each sample. The amount of time which elapsed between the two countings was chosen to allow a large fraction of the shorter-lived isotope to decay without allowing too large a fraction (not more than 0.75) of the longer-lived isotope to decay. A set of two simultaneous equations describes the activity detected during the two countings of a single sample as follows:

\[ X(t_1) = T_A a e^{-\lambda_A t_1} + T_B b e^{-\lambda_B t_1}, \]

\[ X(t_2) = A_0 e^{-\lambda_A t_2} + B_0 e^{-\lambda_B t_2}, \] (16)

where \( X(t_1) \) = total activity in the drops at time = \( t_1 \),
\( T_A \) = transmittance of isotope A through the absorber used,
\( T_B \) = transmittance of isotope B through the absorber used,
\( A_0 \) = activity of isotope A in the drop at time = 0,
\( B_0 \) = activity of isotope B in the drop at time = 0,
\( \lambda_A \) = decay rate constant for isotope A,
\( \lambda_B \) = decay rate constant for isotope B,
and zero time is the time of the intra-arterial injection of the isotopes.

As originally designed, the absorber used in these experiments was a long rectangular sheet of the absorbing material which was pushed along the top of the sample changer slide of the gas-flow counter until it covered the detection chamber. The absorber had to be thin and strong, so a relatively hard alloy of aluminum (Dural No. 1145-H19) was used. In order to choose the best absorber thickness, transmittance curves were obtained by counting various isotopes through various thicknesses of the material. These curves are shown in Fig. 3 for the isotopes $^{42}\text{K}$, $^{32}\text{P}$, $^{22}\text{Na}$, and $^{131}\text{I}$ which were originally chosen for these studies. These curves indicate that a thickness of $0.13 \text{ g/cm}^2$ should give optimal separation of the isotopes. When the absorber was constructed, the original alloy, Dural No. 1145-H19, was not available, so another alloy, Dural No. 1100-H14, was substituted. The absorption of radiation from the four isotopes represented in Fig. 3 was found to be very nearly the same for $0.13 \text{ g/cm}^2$ of both alloys.

When ionic $^{24}\text{Na}$ and colloidal $^{198}\text{Au}$ were chosen as the substances to be injected in these experiments, the absorption of their radiation by the absorber was determined and found to agree with predictions based on a consideration of their $\beta$-particle energies relative to those of the four isotopes studied previously and a consideration of the relative amount and energies of the $\gamma$ radiation from each isotope. Since most of the $\beta$ particles from the $^{24}\text{Na}$ have an energy spectrum with an $E_{\text{max}}$ of $1.394 \text{ MeV}$, and most of the $\beta$ particles from the $^{198}\text{Au}$ have a spectrum with an $E_{\text{max}}$ of $0.29 \text{ MeV}$ (43), more $\beta$ particles from the $^{24}\text{Na}$ than from the $^{198}\text{Au}$ could
Fig. 3. Transmittance curves showing the fractional activity detected from four sources through an absorber of aluminum alloy (Dural No. 1145-H19) as a function of thickness.
penetrate the absorber. Bremsstrahlung, x-rays produced as the velocity of the β particles is decreased by the absorber, and low-energy γ radiation are not greatly affected by the absorber thickness but are detected (with low efficiency due to the small chamber) by the gas-flow detector. This caused the flat tails on some of the absorption curves in Fig. 3. Considering these factors, the previously chosen absorber gave adequate and reasonable differences in intensity loss for ²⁴Na and ¹⁹⁸Au. Therefore, it was not necessary to obtain absorption curves for these isotopes.

Before the experiments requiring the injection of ¹⁴C sucrose were performed, the gas-flow counter was redesigned to allow absorbers of any solid material to be placed over the detection chamber via a slide mechanism. Then an absorber of 0.11 g/cm² of Mylar could be placed over the detector for the first counting of the blood samples containing ¹⁴C. Most of the β particles from ¹⁴C, which have an E_{max} of 0.155 MeV (43), were absorbed by the Mylar absorber, which had very little effect on the higher-energy β particles from ²⁴Na.

The transmittance, T, and decay constant, λ, to be entered into Eqs. (16) have the following values:

for sodium-²⁴ counted through the aluminum absorber,

\[ T = 0.2148, \quad \lambda = 4.605 \times 10^{-2} \text{ hr}^{-1}, \]

for gold-¹⁹⁸ counted through the aluminum absorber,

\[ T = 0.0567, \quad \lambda = 1.073 \times 10^{-2} \text{ hr}^{-1}, \]
for sodium-24 counted through the Mylar absorber,

\[ T = 0.8224, \quad \lambda = 4.605 \times 10^{-2} \text{ hr}^{-1}, \]

for carbon-14 counted through the Mylar absorber,

\[ T = 0.0031, \quad \lambda = 1.420 \times 10^{-8} \text{ hr}^{-1}. \]

These parameters plus the data obtained for each blood sample were
entered on data cards and read into the CDC 6600 computer. Equations (16)
were solved for each sample. As described in the methods section of this
thesis, the correction for recirculation of the isotopes was obtained by
subtraction of the activity concentration of each isotope in the noninjected
leg samples from the activity concentration of the corresponding isotope
in the injected leg samples. Since the blood drops did not fall simulta-
neously from the two sampling catheters, it was necessary to interpolate
the data obtained for the noninjected leg. This was done by the computer
which was programmed to, in effect, draw straight lines between consecutive
data points for the noninjected leg and to obtain from the resulting
straight-line plot of the data the value of the interpolated data for the
noninjected leg at any point in time. Then, from the data for each in-
jected leg sample, the corresponding interpolated data for the noninjected
leg could be subtracted.

A portion of the solution of isotopes to be injected was plated on
the absorbent paper and counted to determine the relative amounts of the
isotopes injected. By use of this information, all data were normalized
to an injection ratio for the two isotopes of 1.0.
Since the β particles from $^{14}C$ have very low energies, it seemed necessary to determine the extent to which self-absorption occurs with the geometry used for the counting of the blood samples dried on filter paper for these experiments.

Some $^{14}C$ sucrose was dissolved in blood, which was then plated on the absorbent paper in 0.01-, 0.02-, and 0.04-ml amounts. Three samples of each size were plated. The samples were dried and then counted. The detected activities from the three samples of each size were averaged and the averages, which exceeded $10^5$ counts in 2 min, were compared. The ratios of these averages were essentially equal to the ratios of the volumes plated. This indicates that self-absorption of the β particles from $^{14}C$ was constant and therefore should not affect the data obtained in these experiments.

Another test for the effect of self-absorption was designed to test the validity of comparing the activity detected from the standard solutions of the isotopes, in which $^{14}C$ is suspended in saline, with the activity detected from the blood samples. Eight 0.02-ml samples of saline containing $^{14}C$ were plated on the absorbent paper. Four of these were covered with a drop of blood which contained no radioactivity. These samples were dried and counted. The average detected activity, which exceeded $10^5$ counts in 2 min, from the blood-covered samples was essentially equal to the average activity from the other four samples. This gives further support of the assumption that it is valid to ignore the self-absorption of $^{14}C$ in these experiments.
RESULTS AND DISCUSSION

A. Intra-Arterial Injection of

Relative Volumes

The net disappearance curves, q(t), for sodium and colloidal gold are shown in Fig. 4 for the control part of experiment #166 and for the same dog under the same physical conditions but during an infusion of 3.03 μg of adrenalin per min per kg of dog. All these curves have been normalized for the amount of each substance injected, q₀. The ratio of the area under the q(t) curve for sodium to that for colloidal gold, the right-hand side of Eq. (6), is 11.4 for the curves of Fig. 4 which were obtained during the control conditions. Therefore,

\[
\frac{V_{\text{control}}}{V_{\text{nonexch}}} = 11.4 \text{ for experiment #166.}
\]

Similar treatment of the curves of Fig. 4 which were obtained during an adrenalin infusion gives

\[
\frac{V_{\text{adren}}}{V_{\text{nonexch}}} = 20.4 \text{ for experiment #166.}
\]

This rather surprising result is in contrast with the result one might expect with adrenalin. As shown below, the adrenalin infusion caused an increased blood flow through the limb. This should cause a decrease in the ratio \( \frac{V_{\text{exch}}}{V_{\text{nonexch}}} \) if the total volume available for exchange remained constant. The observed increase in the ratio reflects
Fig. 4. The net disappearance curves, $q(t)$, for sodium and colloidal gold in the hind limb of the dog after injection into the femoral artery before (control) and during an adrenalin infusion. Time expansions (inserts) of the early portions of the curves show the increase in $q(t)$ as the activity is brought to the tissue via the arterial blood supply. Zero time is the time of injection.
a large increase in the exchange volume.

We can now compute the value of \( \rho \), the ratio of the extravascular tissue-mixing volume, \( V_{tm} \), to the plasma volume, \( V_{pl} \), for each part of the experiment. Equation (9) shows that

\[
\rho = \frac{V_{tm}}{V_{pl}} = k \left( \frac{V_{exch}}{V_{nonexch}} \right) - k,
\]

where \( k \) is the distribution coefficient for \( ^{24}\text{Na} \) between \( V_{pl} \) and \( V_{tm} \). I will let \( V_{tm} \) represent the total extravascular volume, including cells, to which the ionic sodium is distributed. Although one-fifth of the muscle sodium is probably intracellular, I have assumed that most of the injected \( ^{24}\text{Na} \) remains outside the muscle cells viewed by the detectors in these experiments.

In muscle, the exchange between extracellular and intracellular sodium ions is slow. The half-time for this exchange has been reported to be 11 min in rat diaphragm muscle (13), 24 min in frog toe muscle, and 51 min in frog sartorius muscle (26). In my experiments about 95% of the injected \( ^{24}\text{Na} \) washes out of the muscle tissue viewed by the detectors during the first 10 minutes. If we assume a minimum half-time of 30 min for cellular sodium exchange in these tissues, a maximum of 1.5% of the injected sodium can enter these cells in 10 minutes. Therefore, no more than 2% of the injected \( ^{24}\text{Na} \) can enter the cells in the volume of muscle tissue viewed by the detectors in 1 hour. Since the injected \( ^{24}\text{Na} \) remains essentially extracellular during the experiments and since \( V_{tm} \) is to represent the total extravascular distribution volume, including
cells, the average tissue concentration of the extracellular sodium must be used in the determination of the distribution coefficient, \( k \). For ionic sodium in dogs, the average tissue concentration is 32 meq/liter\(^{(42)}\) of which 5.4 meq/liter is probably intracellular sodium. Therefore, the average tissue concentration of extracellular sodium is 26.6 meq/liter of muscle. In dogs, the plasma concentration of ionic sodium is 150 meq/liter \(^{(42)}\). Therefore,

\[
k = \frac{\text{concentration of sodium in plasma}}{\text{concentration of extracellular sodium in tissue}} = 5.65;
\]

hence,

\[
\rho = 5.65 \left( \frac{V_{\text{exch}}}{V_{\text{nonexch}}} \right) - 5.65,
\]

and for experiment \#166, represented in Fig. 4, \( \rho_{\text{control}} \) = 58.9 and \( \rho_{\text{adren}} \) = 109.7. This gives a value of

\[
A = \frac{\rho_{\text{adren}}}{\rho_{\text{control}}} = 1.86.
\]

I have assumed that the intravascular \(^{24}\text{Na}\) is distributed only in the plasma. In order to test this assumption, 5 ml of whole blood from dogs was incubated with 1 ml (50 \( \mu \)Ci) of \(^{22}\text{NaCl}\). After 1 min and 1 hour of incubation, 0.1 ml samples of the whole blood, of the packed blood cells, and of the plasma were counted for \(^{22}\text{Na}\) activity. After 1 min of incubation, 5% of the total \(^{22}\text{Na}\) activity remained with the cell fraction. After 1 hour of incubation, 10% of the total \(^{22}\text{Na}\) activity remained
with the cell fraction.

Since approximately 5% of the total volume of the packed cell fraction is extracellular and would therefore contain plasma, only a negligible amount of the $^{22}$Na ions could have entered the cells during the first minute. After 1 hour, approximately 5% of the $^{22}$Na ions had entered the cells. The intravascular $^{24}$Na ions can therefore be assumed to remain extracellular during the experiments reported in this thesis.

Table I presents the adrenalin infusion rates and the computed values of the relative volumes, $\rho$, and the values of $A$, the fractional change in $\rho$, for six experiments. The value of $A$ is greater than unity in all experiments except #161 and #163, in which low adrenalin infusion rates were used. We can conclude that at sufficiently high dose rates, an adrenalin infusion is accompanied by an increase in the amount of tissue bathed per unit of vascular volume. No conclusion can be drawn at this point concerning the direction of the changes in the respective volumes. These directional changes can be determined if we use the relationship in Eq. (4), to compute the blood flow during the control conditions and substitute this ratio into Eq. (10).

Relative Blood Flows

The curves of the venous blood concentration, $c(t)$, of $^{24}$Na and colloidal $^{198}$Au obtained during the control part of experiment #166 and the corresponding curves obtained during the adrenalin infusion are shown in Fig. 5. All of these curves have been normalized for the amount of each substance injected, $q_o$.

In Fig. 6, the colloidal gold curves from Fig. 5 are shown on a common
Table I. The effect of adrenalin on the values of the relative volumes.

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>Rate of adrenalin infusion (μg/min/kg body weight)</th>
<th>ρ = \frac{V_{tm}}{V_{pl}}</th>
<th>A = \frac{ρ_{adren}}{ρ_{control}}</th>
</tr>
</thead>
<tbody>
<tr>
<td>161 A/B</td>
<td>0.0</td>
<td>58.3</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>0.21\textsuperscript{a}</td>
<td>38.7</td>
<td></td>
</tr>
<tr>
<td>163 A/B</td>
<td>0.0</td>
<td>67.8</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>1.04\textsuperscript{a}</td>
<td>60.4</td>
<td></td>
</tr>
<tr>
<td>164 A/B</td>
<td>0.0</td>
<td>78.0</td>
<td>1.29</td>
</tr>
<tr>
<td></td>
<td>3.03\textsuperscript{b}</td>
<td>100.7</td>
<td></td>
</tr>
<tr>
<td>165 A/B</td>
<td>0.0</td>
<td>43.3</td>
<td>1.55</td>
</tr>
<tr>
<td></td>
<td>3.03\textsuperscript{b}</td>
<td>67.3</td>
<td></td>
</tr>
<tr>
<td>166 A/B</td>
<td>0.0</td>
<td>58.9</td>
<td>1.86</td>
</tr>
<tr>
<td></td>
<td>3.03\textsuperscript{b}</td>
<td>109.7</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a}Infused by intravenous microdrip apparatus. Infusion rate decreased slightly during experiment.

\textsuperscript{b}Infused by motor-driven syringe pump. Infusion rate remained constant.
Fig. 5. The concentration, $c(t)$, of sodium and colloidal gold in the femoral vein of the dog after injection into the femoral artery before (control) and during an adrenalin infusion.
Fig. 6. The concentration, c(t), of colloidal gold in the femoral vein of the dog after injection into the femoral artery before (control) and during an adrenalin infusion, plotted on a common scale to show the difference in the areas under the two curves.

\[
\frac{\text{Flow}_{\text{adrenalin}}}{\text{Flow}_{\text{control}}} = \frac{\text{Area}_{\text{control}}}{\text{Area}_{\text{adrenalin}}} \times \frac{q_0}{q_0^{\text{control}}}
\]
scale. The ratio of the area under the colloidal gold curve obtained during the control conditions to the area under the corresponding curve obtained during the adrenalin infusion gives the value of 8.0 for the relative blood flow rates in experiment #166.

In the third column of Table II, the relative blood flow rates for the five sets of experiments are presented. In experiment #165, the apparent effect of adrenalin on the blood flow was less than in other experiments (#164 and #166) with the same dose of adrenalin. During the adrenalin infusion of experiment #165, the blood sampling rate decreased, probably due to a blood clot in the tubing. The increased transit time for the blood samples in the tubing would cause an increase in the area under the \( c(t) \) curve. This would cause the computed value for the blood flow during the adrenalin infusion to be erroneously low. The relative mean transit times obtained from the in vivo \( q(t) \) curves for experiment #165 agree with the values obtained for experiments #164 and #166 (see Table II), in which the same adrenalin infusion rate was used. Therefore, I have included in Table II the results computed for experiment #165 after replacing the value for the computed relative blood flow with the relative blood flow obtained by averaging the computed relative blood flows for experiments #164 and #166.

**Relative Plasma Volumes**

The directional change in the plasma volume can now be computed. Equation (10) is
Table II. The effect of adrenalin on the vascular volume and on the tissue mixing volume.

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>Rate of adrenalin infusion (\frac{\mu g/min}{kg\ body\ weight})</th>
<th>(\frac{p_{adren}}{p_{control}})</th>
<th>(\frac{t_{adren\ nonexch}}{t_{control\ nonexch}})</th>
<th>(R_p = \frac{v_{adren\ pl}}{v_{control\ pl}})</th>
<th>(R_t = \frac{v_{adren\ tm}}{v_{control\ tm}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>161</td>
<td>0.21(^a)</td>
<td>1.52</td>
<td>1.27</td>
<td>1.93</td>
<td>1.29</td>
</tr>
<tr>
<td>163</td>
<td>1.04(^a)</td>
<td>4.50</td>
<td>0.32</td>
<td>1.46</td>
<td>1.25</td>
</tr>
<tr>
<td>164</td>
<td>3.03(^b)</td>
<td>9.50</td>
<td>0.17</td>
<td>1.61</td>
<td>2.07</td>
</tr>
<tr>
<td>165</td>
<td>3.03(^b)</td>
<td>1.55 (8.75)(^c)</td>
<td>0.21</td>
<td>0.34 (1.89)(^c)</td>
<td>0.52 (2.93)(^c)</td>
</tr>
<tr>
<td>166</td>
<td>3.03(^b)</td>
<td>8.00</td>
<td>0.20</td>
<td>1.86</td>
<td>2.90</td>
</tr>
</tbody>
</table>

\(^a\)Infused by intravenous microdrip apparatus. Infusion rate decreased slightly during experiment.

\(^b\)Infused by motor-driven syringe pump. Infusion rate remained constant.

\(^c\)Values obtained by replacing the relative blood flow computed for experiment #165 (see text) with the average of the relative blood flows computed for experiments #164 and #166.
\[ R_{pl} = \frac{v_{adren}}{v_{control}} = \left( \frac{q_{control}}{q_{nonexch}} \right) \left( \frac{f_0^\infty q_{adren}}{f_0^\infty q_{nonexch}} (t) \, dt \right) \left( \frac{f_0^\infty q_{adren}}{f_0^\infty q_{nonexch}} (t) \, dt \right) . \] (10)

Since all the data have been normalized to equate all values of \( q_o \), the first term in parentheses in the right-hand side of Eq. (10) is unity. The second term, the relative blood flow, has the value of 8.0 for experiment #166. The third term of the equation, the relative mean transit time, is the ratio of the areas under the \( q(t) \) curves of Fig. 4 for colloidal gold during the adrenalin infusion and during the control conditions respectively. The value of this term for experiment #166 is 0.195. The resulting value of \( R_{pl} \) for experiment #166 is

\[ R_{pl} = (8.0) \times (0.195) = 1.56 . \]

The plasma volume increased by a factor of 1.56.

**Relative Mixing Volumes**

The ratio of the extravascular tissue mixing volumes is

\[ R_{tm} = \frac{v_{adren}}{v_{control}} = R_{pl} \cdot A . \]

For experiment #166,

\[ R_{tm} = (1.56) \times (1.86) = 2.90 . \]

The extravascular tissue mixing volume for ionic sodium increased by a factor of 2.90.

The effect of the adrenalin infusion on the arterial pressure in
experiment #166 is shown in Fig. 7. The mean arterial pressure increased from 133 mm Hg during the control run to 207 mm Hg during the adrenalin infusion, an increase of 56%. The increased intra-arterial pressure suggests a possible increase in filtration, which could account for part of the observed increase in the extravascular mixing volume.

The data recorded in my experiments allow no direct correlation between intracapillary and intra-arterial pressures. The adrenalin infusion decreases the effect of diffusion limitation on the clearance of sodium from the plasma (see next section of this thesis). This suggests a decrease in the blood flow through individual capillaries which would reflect, according to Zwiefach (50), constriction of the metarterioles or precapillary sphincters or both. If this occurs, the adrenalin-induced increase in filtration would be less than that predicted by the change in the arterial pressure, if filtration increases at all.

On the other hand, filtration across the capillary walls of all the intravascular fluid could account for only a 25% increase in the volume of the extravascular-extracellular space of the whole animal, which is about 10% of the change observed in the leg during these experiments. The upper limit for filtration would be less than this value because of the increased colloid osmotic pressure in the plasma and the increased elastic forces in the tissue, which would eventually balance the increased hydrostatic pressure.

The values of $R_{pl}$ and $R_{tm}$ and the relative blood flow and the relative mean transit times for the five sets of experiments are listed in Table II. These data, with the possible exception of experiment #165 (discussed earlier), indicate that, in muscle, the extravascular mixing volume for
Fig. 7. The effect of the intravenous adrenalin infusion on the arterial pressure of the dog represented in Figs. 4, 5, 6, and 8.
sodium increases by as much as 190% when adrenalin is infused into the dog. This means that as much as 65% of the resting muscle tissue is not nutritively perfused with blood and that under certain conditions, this nonperfused tissue can become perfused. Nonperfused tissue would soon become ischemic, but the observations of Zwiefach and Metz (52) suggest the existence of a dynamic condition in which the capillaries open and close. This condition could be controlled by some feedback mechanism so that a feedback-controlled fraction of the tissue is nonperfused but no single region of the tissue remains nonperfused very long. A possible mechanism could involve control of the proportionate time spent in stasis and in flow by blood in the capillaries. This could result from a change of threshold to whatever stimuli cause the stasis and the flow.

**Effective Shunts**

In the theory section of this thesis, I demonstrated that the curve of $R_c(t)$, where $R_c$ is the ratio of the ionic $^{24}\text{Na}$ concentration to the colloidal $^{198}\text{Au}$ concentration in the blood samples, can give qualitative evidence for the presence of a shunting mechanism in skeletal muscle. The curve of $R_c(t)$ is shown in Fig. 8B for data collected during the adrenalin infusion of experiment #166 and in Fig. 8A for data collected before that adrenalin infusion. The early dip in the curve in Fig. 8A can be interpreted by assuming the presence of both capillaries and a "shunting mechanism" as described in the theory section of this thesis. Similar curves were obtained for 11 of 16 control experiments. Yudilevich and Friedman (personal communication) have obtained similar results using ionic rubidium-86 as the test substance in perfused skeletal muscle.
Fig. 8. The ratio, $R_c(t)$, of sodium to colloidal gold in the femoral vein of the dog after injection into the femoral artery before (control) and during an adrenalin infusion.
preparations. The failure to obtain similar curves in all the control experiments could indicate that "shunting mechanisms" do not always exist in resting muscle. The level of anesthesia, and the temperature of the dog (which I attempted to keep constant, but occasionally failed), have not been investigated as factors affecting the extent to which shunting exists in resting skeletal muscle.

The \( R_c(t) \) curve obtained during the adrenalin infusion, Fig. 8B, contains no dip in the early region and is similar to the theoretical curve in Fig. 2A. This indicates that the adrenalin infusion was accompanied by a decrease in the effect of the "shunting mechanism." Similar results were obtained in all the five experiments which exhibited the presence of shunts during the control run.

B. Intra-Arterial Injection of \( ^{24}\text{NaCl} \) and \( ^{14}\text{C Sucrose} \)

The time function, \( R_c(t) \), of the ratio of the concentration of \( ^{24}\text{Na} \) to \( ^{14}\text{C sucrose} \) in the venous blood for two experiments is shown in Fig. 9. The upper curves represent data collected from one dog during consecutive runs and the lower curves represent data collected from a second dog during consecutive runs. Three of these curves exhibit a relatively flat early portion followed by a rising portion. The flat early portions of these 3 curves show no loss of \( ^{24}\text{Na} \) relative to \( ^{14}\text{C sucrose} \). If the clearance of either substance is diffusion-limited, sodium should leave the intravascular space more rapidly than sucrose, since sodium ions diffuse nearly 3 times as fast as sucrose in water (10,33).

The rising portions of the \( ^4R_c(t) \) curves in Fig. 9 occur at a
Fig. 9. The ratios, $R_c(t)$, of sodium to sucrose in the femoral vein of the dog after injection into the femoral artery before (control) and during muscle stimulation (upper curves) and before (control) and during an adrenalin infusion (lower curves).
time when the concentrations of both labeled substances in the venous blood are decreasing. This indicates that the $^{24}\text{Na}$ returned to the intravascular space more rapidly than the $^{14}\text{C}$ sucrose after the peak concentration of the substance had passed through the vascular bed. Therefore, the $^{24}\text{Na}$ in the extravascular space was more concentrated than the $^{14}\text{C}$ sucrose after the peak had passed. It follows that the $^{24}\text{Na}$ was cleared from the blood more rapidly than the $^{14}\text{C}$ sucrose during the time when the concentrations of these substances were increasing in the blood within the capillaries. The early samples of the activity which passed through the capillaries would have a value of $R_c$ that is less than unity. These samples must have reached the venous sampling site at about the same time as the major portion of the effectively shunted activity for which $R_c$ would be unity. Then, if the value of $R_c$ deviated from unity by a small amount in the capillary blood appearing in each sample, this deviation could be masked by the large amount of shunted activity in each sample that would have a value of $R_c$ equal to unity.

The one curve in Fig. 9 which does not exhibit the flat early portion was obtained during an adrenalin infusion. That curve shows an early loss of $^{24}\text{Na}$ relative to $^{14}\text{C}$ sucrose, since the value of $R_c$ was slightly less than unity in some of the early samples. This suggests a decrease in the masking of the early low values of $R_c$ which, in turn, suggests that these samples contained relatively less effectively shunted blood than the corresponding samples collected during the other three runs. This result with adrenalin is in agreement with the results of similar experiments, reported earlier in this thesis, in which $^{24}\text{Na}$ and colloidal $^{198}\text{Au}$ clear-
ances were studied. In those experiments, adrenalin infusions were accompanied by a decrease in the effect of the shunts on the total clearance of the labeled substances.

C. Arterial Occlusion

Experiment #162 was originally designed to be the same as the other paired intra-arterial injections of $^{24}$NaCl and colloidal $^{198}$Au described in the methods section of this paper. Immediately after the injection of the isotopes, during the adrenalin infusion, a temporary supra-arterial pressure was applied in the inguinal region over the femoral artery of the injected leg. This was done to facilitate removal of the injection syringe from the tubing connected to the intra-arterial needle. The results of this error gave additional information supporting the previously presented interpretation of the $R_c(t)$ curves.

The curves of the venous blood concentration, $c(t)$, of $^{24}$Na and colloidal $^{198}$Au obtained during both the control and the adrenalin infusion-arterial occlusion parts of experiment #162 are shown in Fig. 10. If the blood flow, $F$, is constant, each curve of Fig. 10 is the frequency function of the transit times required for particles of the injected substance to travel from the injection site to the sampling site. The frequency function, $h(t)$, of transit times for a unit injection and unit blood flow could be obtained by multiplying the function $c(t)$ by the blood flow, $F$, divided by the amount of the substance injected, $q_o$. If $F$ or $q_o$ is not known, $h(t)$ can be obtained by normalizing $c(t)$ to set its integral equal to unity.
Fig. 10. The concentration, \( c(t) \), of sodium and colloidal gold in the femoral vein of the dog after injection into the femoral artery before (control) and during a combination of the adrenalin infusion and a brief arterial occlusion.
The \( c(t) \) curves often exhibit deviations from the skewed normal distribution function which might be expected if the transit time required for a particle to traverse the capillary bed is a continuous random variable. The \( c(t) \) curves obtained for experiment \#162, shown in Fig. 10, exhibit very pronounced deviations which make it easy to visualize the several components which form the total curve. In Fig. 11 the \( c(t) \) curves for \(^{24}\text{Na} \) from experiment \#162 appear as broken lines. Solid lines have been drawn to show the possible components which could be summed to give the total \( c(t) \) curve. Each of these components would then represent the frequency function of transit times for the \(^{24}\text{Na} \) ions which pass through one element of the vascular bed which has characteristics that make it distinguishable from the other element(s) of the vascular bed. Zierler (49) has described the resulting over-all frequency function of transit times that would occur when two elements, shunts and capillaries, occur in parallel. Additional elements must be considered if shunts of different lengths or at different distances from the injection site can be distinguished or if capillaries that perfuse different volumes of tissue can be distinguished by different distributions of transit times.

If the values of the modal transit time (the peak of the frequency function of transit times) for particles in each of the elements are nearly equal, the resulting \( c(t) \) curve would have a single peak and could be smooth enough to give no indication of its separate components. Conversely, if the values of the modal transit time for each element are quite different, the resulting \( c(t) \) curve could have several peaks, each
Fig. 11. Components (solid lines) of the curves of $c(t)$ (broken lines) for sodium, taken from Fig. 10.
corresponding to the peak of the distribution function of mean transit times for particles in one of the elements.

The c(t) curves in Fig. 10 show that in experiment #162, under control conditions, the modal transit times were sufficiently different in several of the elements to show four separate peaks. The effect of the combined adrenalin infusion and arterial occlusion was to increase all the modal transit times so that the separate components of the c(t) curve became even more evident.

After an intra-arterial injection of $^{131}$I albumin, Baker (5) obtained venous c(t) curves with more than one peak. He suggested that the second peak represented a second circulation, possibly shunts opened when he increased the perfusion pressure. I believe it equally possible that his increasing the perfusion pressure merely caused an increase of the difference in the modal transit times of the separate elements already present.

Assuming that the separate components of the c(t) curve do represent separate elements of the vascular bed, the time function, $R_C(t)$, of the ratio of the $^{24}$Na concentration to the $^{198}$Au concentration in the venous blood can now be interpreted in terms of these elements.

The early samples of activity from an element composed of capillaries should be less than unity because of the loss of $^{24}$Na to the extravascular space. After the peak activity passes through the element, the value of $R_C$ should become greater than unity as the extravascular $^{24}$Na returns to the intravascular space. These changes have been discussed in this thesis under the heading "The Theory of Effective Shunts."

If the arrival of activity from another element composed of capillaries
prevents the value of $R_c$ from becoming greater than unity, it follows that the values of $R_c$ from that element are low enough to mask the higher $R_c$ values in the activity from the previous elements.

Since the components of the $c(t)$ curve are frequency functions of transit times for particles traversing separate elements of the vascular bed, each component reflects a longer mean transit time, $\bar{t}$, than the previous component. Remembering that $\bar{t} = V/F$, we see that subsequent components of the $c(t)$ curve reflect elements of the vascular bed which have larger mixing volumes, $V$, or smaller blood flows, $F$, (or both) than the previous elements. Larger mixing volumes indicate either larger extravascular perfusion volumes or larger capillary volumes or both. The larger extravascular perfusion volumes would account for a larger loss of $^{24}\text{Na}$ relative to the intravascular $^{198}\text{Au}$, hence a smaller value of $R_c$ in the early samples from a given element. A smaller blood flow through a given element could also account for a larger loss of $^{24}\text{Na}$ relative to $^{198}\text{Au}$ if the clearance of $^{24}\text{Na}$ from the plasma is significantly diffusion-limited.

The $R_c(t)$ curves for experiment #162 are presented in Fig. 12. The corresponding components of the $c(t)$ curves for $^{24}\text{Na}$, taken from Fig. 11, have been superimposed as broken lines to show the time relationships between these components and the changes in the $R_c(t)$ curve.

During the control part of experiment #162, the ratio, $R_c$, increased slowly at first and then rapidly. The samples which indicate a slow change in $R_c$ were collected when a large portion of the sampled activity had come through the first element, represented by component #1 of the $c(t)$ curve.
Fig. 12. The ratio, $R_c(t)$, of sodium to colloidal gold in the femoral vein of the dog after injection into the femoral artery before (control) and during a combination of an adrenalin infusion and a brief arterial occlusion. The components of the $c(t)$ curve for $^{24}\text{Na}$, from Fig. 11, are included.
As more activity arrived from the second element, component #2 of the c(t) curve, the value of $R_c$ increased more rapidly. These changes in $R_c$ can be explained by assuming that the first element of this vascular bed is composed of shunts, either "anatomical" or "effective" or both, and that the second element is composed of capillaries.

Some of the shunts in the first element must be very short or must have a very high linear blood flow, since activity appeared at the venous sampling site very quickly after the injection. The time between injection into the artery and appearance of the activity at the sampling site is consistently 1 second plus or minus approximately 1 second.

The value of $R_c$ increased most rapidly at the time when most of the activity appearing in the blood samples is activity which passed through the second element. This time is indicated by the peak of component #2 of the c(t) curve. When the activity that passed through the third element appears in the samples, it causes a flattening of the $R_c(t)$ curve. Therefore, the third element is another group of shunts. Since the peak activity from the third element accounts for only about one half of the total activity arriving at that time, the value of $R_c$ for the total activity is not decreased to unity. The activity from the third element washes past the sampling site very rapidly, allowing the activity from the second element to become slightly more significant and therefore raising the value of $R_c$.

When the peak activity from the fourth element, another group of shunts, arrives, it represents a very significant portion (87%) of the total activity and causes the value of $R_c$ for the total activity to
decrease. The value of $R_c$ becomes less than unity when activity appears from the fifth element, which is therefore another group of capillaries. Then the value of $R_c$ increases as the activity from the second and fifth elements (capillaries) becomes more significant than the activity from the fourth element (shunts). The value of $R_c$ again decreases when the slope of component #5 of the $c(t)$ curve is steeper than the slope of the tail of component #4.

The $R_c(t)$ curve, Fig. 12, for the data obtained after a short arterial occlusion during the adrenalin infusion of experiment #162 indicates that component #1 of the $c(t)$ curve represents an element composed of shunts. The first sample contained mostly, but not completely, shunted activity, which held the value of $R_c$ close to unity. A larger proportion of the second sample of activity came from the second element (capillaries) of the vascular bed, which decreased the value of $R_c$. The third sample was obtained closer to the peak of component #1, and it therefore contained more shunted activity which again brought the value of $R_c$ closer to unity. The next three samples were obtained when component #1 was decreasing rapidly, and so they reflect the increasing value of $R_c$ in the activity from the second element. The value of $R_c$ again decreases as component #3 becomes important. The early samples from the third element, therefore, have an $R_c$ value less than unity, so the third element must be another group of capillaries. The $R_c(t)$ curve begins to rise slightly but again decreases when activity arrives from the fourth element. Then the curve flattens slightly until activity arrives from the fifth element. Therefore, $R_c$ must have been less than
unity in the early samples of activity from the fourth and fifth elements, which indicates that these elements are also groups of capillaries.

The value of $R_c$ did not become greater than unity during the sampling period of this part of the experiment. This indicates that from each subsequent element of the vascular bed a larger fraction of the $^{24}\text{Na}$ left the intravascular space, resulting in a lower value of $R_c$ for the early samples of activity from that element. The value of $R_c$ must eventually become greater than unity, but samples were not obtained at that time.

D. The Validity of Relating the Exponential Components of the Disappearance Curve to the Elements of the Vascular Bed

The results reported in the preceding subsection of this thesis indicated that the peripheral vascular bed in the hind limb of the dog is composed of several elements, each element having perfusion characteristics peculiar to itself. If a substance is present in one of the elements but not in the affluent blood and if the movement of that substance is a result of diffusion and blood flow, no active processes being involved, the substance should leave the element at a rate $dq_i/dt$, which is proportional to the amount of the substance present $q_i(t)$. That is,

$$\frac{dq_i}{dt} = -k_i q_i(t), \quad (17)$$

where the rate constant, $k_i$, is equal to the fractional turnover rate, $F_i/V_i$, of the volume, $V_i$, of the fluid containing the substance. This
fluid is being replaced by the affluent blood, which enters the element at the rate $F_i$. The substance must be essentially equilibrated throughout the volume $V_i$ at all times in order for the definition of $k_i$ in Eq. (17) to be valid. Therefore, Eq. (17) does not apply, as defined, to any substance whose clearance from the element is, to a detectable extent, diffusion-limited.

Suppose a substance whose clearance is not diffusion-limited is injected into the femoral artery of the dog. The substance will be delivered by the arterial blood to all the vascular elements fed by the femoral artery. Then the substance will leave each element at an exponential rate as shown in Eq. (17). The total rate, $dq/dt$, of the activity leaving the entire vascular bed will be the sum of the individual rates for each element present. For a vascular bed containing $n$ elements,

$$\frac{dq}{dt} = \sum_{i=1}^{n} -f_i k_i q_i,$$

(18)

where $f_i$ is the fraction of the injected activity that entered element $i$.

In the past, Eq. (18) has been solved to obtain the function which described the total disappearance curve, $q(t)$ (16,17). The result obtained is

$$q(t) = q_0 \sum_{i=1}^{n} f_i e^{-k_i t},$$

(19)

where $q_0$ is the amount of the substance present at zero time, which is generally assumed to be the time of the injection.

Equation (18) can be valid for a total vascular bed only during the
time when the substance is being washed out of every element within the vascular bed. The substance is being deposited into any given element while the peak concentration of the substance in the blood of that element flows through it. At some time after this peak concentration leaves the element (this is the modal transit time for the element, as discussed in the preceding section of this thesis), the substance leaves the element at the rate described by Eq. (17). Therefore, Eq. (18) is not valid for the total vascular bed until some time after the peak concentration of the substance has left the element with the longest modal transit time. It follows that the total disappearance curve for a vascular bed can be treated as a sum of exponential components which represent separate elements only if the zero time is defined to be at some presently unknown time after the longest modal transit time occurring in the vascular bed.

The results discussed in the preceding subsection of this thesis indicate that essentially all the $^{24}\text{Na}$ that entered the first element had completely left that element before any $^{24}\text{Na}$ appeared from the fourth and fifth elements. When Eq. (18) becomes valid, at least one element is no longer contributing to the $q(t)$ curve. Therefore, for the peripheral vascular bed studied in these experiments, it is not valid to obtain exponential components of the disappearance curve for labeled sodium ions and then assume that these components are related to the physiological elements of the vascular bed. Zierler (47) has discussed this problem in terms of the lagged exponential functions which might describe the rate at which the $^{24}\text{Na}$ leaves each element.
E. The Shapes of the Disappearance Curves

A semilogarithmic plot of the time function of the fraction of the peak amount of injected $^{24}$Na present in the sensitive field of the in-vivo radiation detector is shown as a solid line in Fig. 13 for each part of experiment #166, which was described in an earlier subsection of this thesis (see Results and Discussion A). On these curves and for the discussion in this section of the thesis, zero time is the time of completion of the injection when $q(t)$ begins to decrease. According to the discussion in the preceding section of this thesis, it would not be valid to obtain exponential components of these curves and treat the components as though they represent separate physiological elements of the vascular bed as defined here. However, semilogarithmic plots of the disappearance curve should not be overlooked as a source of some pertinent information about the vascular bed.

If we assume that any diffusion limitation of sodium clearance from the plasma occurs at the capillary wall rather than in the extravascular fluid, the fact that the semilogarithmic plot of $q(t)$ is not a straight line indicates the inhomogeneous nature of the vasculature [the presence of separate regions which have various degrees of perfusion (16,17,36,37)] in the tissues within the sensitive field of the detector. The steep initial slope of the curve reflects the large amount of activity that entered the more highly perfused regions of the vascular bed. In spite of the fact that the relative weights may not be determinable for the separate vascular elements involved in the early portion of the $q(t)$
Fig. 13. Semilogarithmic plots of the fractional disappearance curves for $^{24}\text{Na}$ in the hind limb of the dog after injection into the femoral artery before (control) and during an adrenalin infusion. Zero time is the time the peak occurred. The initial slope and the extrapolation of the tail portion of the curves are shown by broken lines.
curve, the slope of that portion of the curve represents a weighted average rate of perfusion of the more highly perfused regions of the vascular bed.

The tail of the semilogarithmic plot of \( q(t) \) is a straight line in all the nineteen curves which have been plotted in this manner. This tail is a single exponential, which suggests that it does represent a single element of the vascular bed. It is logical to assume that at some point in time, the \( ^{24}\text{Na} \) in all but the most poorly perfused element would be washed from the leg. Then it follows that the slope of the tail of the \( q(t) \) curve is the rate constant for the exponential loss of \( ^{24}\text{Na} \) from the remaining element. In addition, the fractional zero-time intercept of the extrapolated tail portion of the curve represents the fraction of the total injected \( ^{24}\text{Na} \), thus the fraction of the leg blood flow, that entered the most poorly perfused element, since entry of the \( ^{24}\text{Na} \) into this element is probably fast relative to removal.

We now have three parameters which can be used to describe the early and late portions of any single \( q(t) \) curve. These parameters can then be used to compare pairs of groups of curves.

The difference between the two \( ^{24}\text{Na} \) curves obtained during experiment #166 are more obvious in the semilogarithmic plots of Fig. 13 than in the rectilinear plots of Fig. 4. The broken lines in Fig. 13 represent the slope of the initial portion and the extrapolation to zero time of the tail portion of each curve, which were used to obtain the values of the three parameters discussed above.

Table III lists the values of the parameters obtained from the \( q(t) \)
Table III. The effect of adrenalin infusion on the values of three parameters of the semilogarithmic plot of q(t) for $^{24}\text{Na}$.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Adrenalin Inf.</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Exp.No.</td>
<td>Value</td>
</tr>
<tr>
<td>Initial Slope</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(min$^{-1}$)</td>
<td>146</td>
<td>5.1</td>
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<tr>
<td></td>
<td>147</td>
<td>58.7</td>
</tr>
<tr>
<td></td>
<td>152B</td>
<td>182.5</td>
</tr>
<tr>
<td></td>
<td>153B</td>
<td>72.9</td>
</tr>
<tr>
<td></td>
<td>155A</td>
<td>61.3</td>
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<tr>
<td></td>
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<td>98.8</td>
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<td></td>
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<td>13.2</td>
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<tr>
<td></td>
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<tr>
<td></td>
<td>165A</td>
<td>81.5</td>
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<tr>
<td></td>
<td>166A</td>
<td>27.7</td>
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<tr>
<td>Limits of p</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final slope</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(min$^{-1}$)</td>
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<td>0.023</td>
</tr>
<tr>
<td></td>
<td>147</td>
<td>0.051</td>
</tr>
<tr>
<td></td>
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<tr>
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<td>163A</td>
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<td></td>
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<td>0.035</td>
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<tr>
<td>Average</td>
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<tr>
<td>Standard deviation</td>
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<tr>
<td>Fractional zero time</td>
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<tr>
<td>Standard deviation</td>
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<tr>
<td>Difference</td>
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<td></td>
</tr>
<tr>
<td>Limits of p</td>
<td></td>
<td></td>
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curves of 10 experiments. Five of these experiments included an adrenalin infusion as described in the methods section of this thesis. For the other 5 experiments, only the control parts are being reported here. The data in Table III are divided into two groups, the 10 control parts and the 5 adrenalin-infusion parts of the experiments. For each parameter, the arithmetic average of each group (sample) was obtained. Then these averages were tested to determine whether or not the adrenalin infusion caused a significant change in that parameter.

In order to test the significance of the difference in the two averages obtained for a given parameter, the assumption is made that the true means of large samples of each group (controls and adrenalin infusions) should be equal, i.e., an adrenalin infusion causes no change in the parameter. Then the t test (22) can be applied to obtain the limits of the probability, $p$, that a difference in the averages which is larger than the observed difference would occur due to random sampling error. A small value of $p$ would indicate a large probability that the observed difference in the averages is a result of the adrenalin infusion rather than random sampling error.

Table III lists the averages and the standard deviations for the two groups of values of each of the parameters. The difference in the averages for the two groups and the value of $p$ for that difference are also shown in Table III.

The results shown in Table III indicate that adrenalin increases the initial slope of the semilogarithmic plot of $q(t)$ by more than a factor of two. This change indicates that adrenalin significantly (the value of
p is less than 0.05) increases the rate of perfusion in the more highly perfused regions of the tissues in the detection field. On the other hand, the small increase in the final slope and the higher value of p (0.15 < p < 0.20) indicates that adrenalin causes little, if any, change in the rate of perfusion in the most poorly perfused element of the vasculature within those tissues. The change in the average fractional zero-time intercept of the extrapolated tail portion of the q(t) curve indicates that adrenalin very significantly (the value of p is less than 0.005) decreases (by a factor of 0.5) the fraction of the leg blood flow that enters the most poorly perfused element. This reflects an increase (by a factor of 1.05) in the fraction of the leg blood flow that enters the more highly perfused regions.

The large standard deviations shown in Table III reflect the high variability of the values of the parameters obtained for different dogs. Of the 3 parameters, the initial slope is the most variable under control conditions. This is also the parameter which shows the greatest change when adrenalin is infused. It is interesting to note that the standard deviation, which was 73% of the average value in the control group, was decreased to 54% of the average value in the adrenalin group. Considering that the adrenalin group was a smaller sample and that different rates of adrenalin infusion were used (the rates of adrenalin infusion are shown in Table II), the decreased standard deviation seems very significant. This decrease suggests that the infusion of adrenalin removes, or at least decreases, some physiological variables in the vascular bed, i.e. the adrenalin tends to clamp, by maximization, certain physiological responses.
such as increased blood flow in some region(s).

The final slope of the \( q(t) \) curve is not so variable in the control group as the initial slope, and the effect of adrenalin on the final slope is very small or nonexistent. This suggests that the rate of perfusion of the poorly perfused region of the tissue is not a very significant physiological variable. Prentice et al. (34) reported similar results with isolated, perfused skeletal muscle tissue. On the other hand, adrenalin causes a significant change (by a factor of 0.5) in the fraction of the injected activity that enters this poorly perfused region. Since the fraction of the activity entering a region reflects the fraction of the total blood flow entering that region, and considering that the average change in the total blood flow with adrenalin is greater than a factor of 4 (see Table II), the blood flow through the poorly perfused regions must increase with adrenalin. Therefore, it seems that the blood flow in the most poorly perfused element of the vascular bed is a significant physiological variable. Since the rate of perfusion, \( F/V \), in this element of the vascular bed is fairly constant, the total volume of this element must also be a significant variable.
CONCLUSIONS

In the resting, anesthetized dog, a large fraction of the muscle tissue in the hind limb is not nutritively perfused with blood. When adrenalin is infused into the dog, the volume of nutritively perfused tissue, \( V_{tm} \), can be increased to three times the original volume. If the adrenalin infusion causes all the tissue to become nutritively perfused, the increase by a factor of 3 indicates that two-thirds of the tissue was not nutritively perfused prior to the infusion. If the adrenalin does not cause all the tissue to become nutritively perfused, then a larger fraction than two-thirds of the tissue had not been nutritively perfused.

The existence of any tissue which is not nutritively perfused suggests the presence of effective shunts, which can include anatomical shunts and other shunting mechanisms, or the presence of diffusion barriers which divide the total exchange volume into many smaller volumes. These smaller volumes could be groups of muscle fiber bundles which, according to the description of the vasculature in muscle given by Zwiefach and Metz (52,53) might be invested only by a group of capillaries from a single arteriole.

The values of the ratio, \( R_c \), of \( ^{24}\text{Na} \) to colloidal \( ^{198}\text{Au} \) in the venous blood samples obtained immediately after the intra-arterial injection of the substances also support the assumption that effective shunts exist in the tissues studied. The data indicate that the effectiveness of the shunts is decreased when adrenalin is infused into the dog. Similarly, after an intra-arterial injection of \( ^{24}\text{Na} \) and \( ^{14}\text{C} \)
sucrose, the loss of $^{24}\text{Na}$ relative to $^{14}\text{C}$ sucrose was probably masked in the early venous blood samples by large amounts of the labeled substances which passed through effective shunts. When adrenalin was infused into the dog, the loss of $^{24}\text{Na}$ became slightly more apparent due to the decreased effectiveness of the shunts. This decrease in the effectiveness of the shunts with an adrenalin-induced increase in the blood flow is in contrast with the effect of mechanically increasing the perfusion pressure in a perfused muscle preparation. Baker (5) has reported an increase in the effectiveness of the shunts during such manipulations. This suggests that the increased perfusion pressure merely forced proportionately more blood through the channels with the lowest resistance, the shunts, but adrenalin opens proportionately more capillaries, so that the flow through the shunts does not increase so much as the flow through the capillaries.

The results of several experiments reported in this thesis support the assumption that the observed effective shunts in skeletal muscle tissue could actually include both anatomical shunts and various degrees of diffusion limitation on the clearance of $^{24}\text{Na}$ from the plasma in that tissue. The relationship between the functions $R_c(t)$ and $c(t)$ in the venous blood leaving the tissue after an intra-arterial injection of $^{24}\text{NaCl}$ and colloidal $^{198}\text{Au}$ during an arterial occlusion and adrenalin infusion strongly suggests that no $^{24}\text{Na}$ left the plasma that flowed through the first element of the vascular bed. This indicates that the first element was composed of anatomical shunts. Zwiefach and Metz (52) and Saunders (39) have reported seeing anatomical shunts in skeletal muscle. The results of studies of the effect of lumbar sympathectomy
on the circulation in the human calf and toe by Rapaport et al. (35) suggest that shunt vessels, which exhibit sympathetically controlled tone, exist in skeletal muscle. The presence of some degree of diffusion limitation on the clearance of $^{24}\text{Na}$ from the plasma is indicated by the greater loss from the plasma of $^{24}\text{Na}$ than of $^{14}\text{C}$ sucrose in all four experiments in which these two substances were injected. Jones (25) has shown that the clearance of gases from the plasma in skeletal muscle tissue is not diffusion-limited, but Friedman and Yudilevich will soon publish data indicating that the clearance of some ionic species is partially diffusion-limited. Crone (14) has shown that sucrose clearance in skeletal muscle is partially diffusion-limited, and Lassen (29) has shown that, during reactive hyperemia, clearance of intramuscularly injected $^{24}\text{Na}$ is partially diffusion-limited.

There are two possible responses which could explain how adrenalin decreases the effectiveness of the shunts. The anatomical shunts could become closed to the circulation in response to the adrenalin infusion. Weideman (46) has stated that, according to the recent literature, blood flow through arter-venous anastomases could be under direct humoral control, but Zwiefach (50) and Zwiefach and Metz (5) have suggested that the flow through the preferential channels, or shunts, which have a low but constant resistance, varies passively as the capillary flow is varied by different vasoactive stimuli. Green and Kepchar (20) have reviewed the observations of several investigators who suggest that epinephrine exerts both a constrictor and a dilator effect on different vessels within skeletal muscle vascular beds. Honig and Gabel (23) reported that
epinephrine and norepinephrine cause a redistribution of blood flow between nutrient and nonnutrient channels, as determined in their oxygen tension studies. The work of Barány (6) suggests similar changes with adrenalin and noradrenalin. Adrenalin could also decrease the effectiveness of the shunts by opening capillaries which were previously closed. This response would change the effectiveness of the shunts in two ways. It would decrease the fraction of the muscle blood flow that passes through the anatomical shunts and it would decrease the physical distance between capillaries, allowing diffusion equilibrium to occur more rapidly. This single response could therefore simultaneously decrease the effectiveness of both the shunting mechanisms that have been suggested.

Evidence for nonuniform changes in the distribution of the blood flow through the tissues in the sensitive field of the radiation detector is found in the semilogarithmic plots of the time function, q(t), of $^{24}\text{Na}$ in the tissues. From these plots, we see that an adrenalin infusion causes very little change in the rate of perfusion (flow/volume) in the most poorly perfused region of the tissue and that it causes a large increase in the blood flow through that region. This indicates that there is an increase in the volume of poorly perfused tissue during an adrenalin infusion. The plots also indicate that the perfusion rate in the more highly perfused regions increases by a larger factor than the fraction of the leg blood flow that enters those regions. Therefore, during an adrenalin infusion, the volume of highly perfused tissue is increased by a smaller factor than the volume of poorly perfused tissue.

The combined results of all the experiments reported in this thesis
suggest that, when adrenalin is infused into the dog, previously closed capillaries become open. Some of these newly opened capillaries are in regions of tissue which were previously not nutritively perfused. The resulting spatial distribution of open capillaries is not homogeneous, since some regions of the tissue are more highly perfused than other regions. When compared with the preinfusion distribution, which was also nonhomogeneous, the new distribution of open capillaries reflects a smaller increase in the volume of the highly perfused tissue than of the poorly perfused tissue. Adrenalin, therefore, causes nonuniform changes in the nonhomogeneous distribution of capillaries in the skeletal muscle tissues. None of the results of these experiments precludes the possibility that adrenalin also causes the closing of some anatomical shunts.

The variability of the number of open capillaries in skeletal muscle tissue would affect the values obtained by Crone (15) and by Pappenheimer et al. (33) for the permeability of the capillary membrane, but the data from their experiments give no measure of what fraction of the total number of capillaries was open. This variability also suggests that the values of $S/V_t$ quoted by Morales and Smith (32) were erroneously high, which gives additional support to their statement that, in many cases, the von Schrütter approximation gives a poor description of inert metabolite uptake by tissue.

The rapid appearance of a labeled substance at the venous sampling site within about 1 second after the intra-arterial injection of the substance suggests that some of the channels through which the substance can pass are very short and direct and that the blood flow through some of
these short channels must be very rapid.

The tissue perfusion rate in skeletal muscle is extremely variable. The theory and techniques reported in this paper allow us to demonstrate some dynamic aspects of the microcirculation in skeletal muscle that play an important role in the variations of the tissue perfusion rate. The opening of more capillaries and the possible closing of anatomical shunts during an adrenalin infusion increases the perfusion efficiency of the blood bathing the muscle. This decreases the requirements for an increased cardiac output. Exercise, hypovolemic shock, and temperature change may also cause changes in the distribution of the microcirculation in skeletal muscle, which can be demonstrated with similar techniques.
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