Recent Work

Title
MICROSPHERE TRACER STUDIES OF PERIPHERAL VASCULAR SHUNTS

Permalink
https://escholarship.org/uc/item/02h7d0cm

Author
Schmidt, Charles Thomas.

Publication Date
1972-11-01
MICROSPHERE TRACER STUDIES OF PERIPHERAL VASCULAR SHUNTS

Charles Thomas Schmidt  
(Ph. D. Thesis)  
DONNER LABORATORY  

November 1972  

Prepared for the U.S. Atomic Energy Commission under Contract W-7405-ENG-48  

For Reference  
Not to be taken from this room
DISCLAIMER

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor the Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or the Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or the Regents of the University of California.
Microsphere Tracer Studies of Peripheral Vascular Shunts

ABSTRACT

Charles Thomas Schmidt

Measurement of shunt blood flow fraction in the dog's leg has been accomplished by arterial infusion of microspheres (MSP). Using external radiation detectors to monitor the number of radioactive MSP bypassing the leg and reaching the lung, we find that the bypass fraction of MSP is constant in time at least for periods of 8-12 minutes. Additionally the local accumulation rate of MSP in gross areas of the leg such as thigh and paw is also constant. The observed bypass fractions for 15 μ MSP in the normal anesthetized dog ranged from 33% to 93%.

The infusion technique is valuable in following the response of the shunt flow to physiological manipulations since both global and local variations are measured continuously. We have observed the changes in shunt flow caused by epinephrine infusion. The effects are quite striking. Overall leg shunting decreases greatly, sometimes to zero. Local nutritive flow as determined by leg accumulation of MSP also is changed; in the paw nutritive flow decreases while in the muscular thigh region nutritive flow increases.

It is generally accepted that epinephrine reduces skin blood flow and increases muscle flow. The presence of large diameter arterio-venous anastomoses in skin is also well known; our work indicates that few if any such anatomical shunts exist in muscle tissue. Our results with epinephrine are consistent with the above facts and thus support the hypothesis that MSP shunting corresponds
to non-nutritive shunt blood flow.

The anatomical location of the shunts was studied in several ways. When all paw circulation was occluded with a pressure cuff, overall shunt flow decreased, but not as drastically as with epinephrine infusion. Since epinephrine acts on the entire leg while the cuff affects only the paw, this result implies that significant shunting occurs somewhere in the upper leg, as well as in the paw.

The fact that this upper leg shunting is primarily associated with skin was shown in an experiment where all arteries not accepted as supplying only muscle were ligated before MSP infusion. The shunt fraction in this case was only 1.5% compared to the usual shunt range of about 30 to 90%. This observation shows that most if not all shunting of MSP occurs in skin and paw and very little in muscle.
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Introduction</td>
<td>1</td>
</tr>
<tr>
<td>A. General statements on peripheral blood flow</td>
<td>1</td>
</tr>
<tr>
<td>B. Organization and scope</td>
<td>5</td>
</tr>
<tr>
<td>II. Physiology of peripheral circulation</td>
<td>6</td>
</tr>
<tr>
<td>A. General characteristics of peripheral blood flow</td>
<td>6</td>
</tr>
<tr>
<td>B. Response to exercise</td>
<td>10</td>
</tr>
<tr>
<td>C. Response to temperature</td>
<td>12</td>
</tr>
<tr>
<td>D. Response to epinephrine</td>
<td>14</td>
</tr>
<tr>
<td>III. Nutritional blood flow measured with diffusible ions</td>
<td>15</td>
</tr>
<tr>
<td>A. Washout methods</td>
<td>15</td>
</tr>
<tr>
<td>B. Extraction methods</td>
<td>18</td>
</tr>
<tr>
<td>IV. Microsphere studies of blood flow</td>
<td>19</td>
</tr>
<tr>
<td>V. Materials and methods</td>
<td>23</td>
</tr>
<tr>
<td>A. Animal preparation</td>
<td>23</td>
</tr>
<tr>
<td>B. Microsphere characteristics</td>
<td>24</td>
</tr>
<tr>
<td>1. Infusion technique</td>
<td>27</td>
</tr>
<tr>
<td>C. Data collection</td>
<td>29</td>
</tr>
<tr>
<td>D. Data analysis</td>
<td>31</td>
</tr>
<tr>
<td>VI. Results and discussion</td>
<td>36</td>
</tr>
<tr>
<td>A. Magnitude of shunt flow and its constancy with time</td>
<td>36</td>
</tr>
<tr>
<td>1. Comparison of infusion and injections</td>
<td>39</td>
</tr>
<tr>
<td>B. Response of shunt flow to epinephrine</td>
<td>44</td>
</tr>
<tr>
<td>C. The effect of microsphere diameter</td>
<td>50</td>
</tr>
</tbody>
</table>
Table of Contents (cont.)

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. Relative shunt flow in muscle and skin</td>
<td>51</td>
</tr>
<tr>
<td>Summary</td>
<td>58</td>
</tr>
<tr>
<td>Acknowledgments</td>
<td>60</td>
</tr>
<tr>
<td>Appendix I - Weighted Linear Least Squares Fitting</td>
<td>61</td>
</tr>
<tr>
<td>Appendix II - Computer program listings</td>
<td>65</td>
</tr>
<tr>
<td>Appendix III - System dead time measurements</td>
<td>70</td>
</tr>
<tr>
<td>References</td>
<td>73</td>
</tr>
</tbody>
</table>
I. Introduction

I-A. General statements on peripheral blood flow

Peripheral blood flow to the limbs of mammals is primarily concerned with blood supply to skeletal striated muscle and to skin. These two tissues differ considerably in their uses and requirements of blood. Muscle during exercise has a much higher nutritional or metabolic requirement than resting muscle and hence muscle blood supply should change in response to this need. The nutritional requirements of skin on the other hand remain relatively constant. In man, changes in skin blood flow can arise from the requirements of whole body and/or local thermoregulation. Exposure of the body to cold results in decreased skin blood flow. Thus although the temperature gradient across the skin has increased, the change in skin blood flow reduces heat loss and acts in homeostatic defense of the internal body temperature. According to Bullard (1966), an interesting example of these two reactions occurs during exercise. Initially skin blood flow decreases as some of the skin flow is shifted to supplying the contracting muscle. As the exercise is continued, body temperature starts to rise and skin blood flow increases in order to dissipate the exercise induced heat.

Returning to the phenomenon of changes in skin blood flow in response to external temperature changes, let us consider how this may be accomplished physiologically. The temperature stimulus causes little change in the metabolic requirements of the skin. Thus it is reasonable to assume, and indeed it is well documented,
that some skin blood flow never encounters a capillary as it
passes from artery to vein (Greenfield (1962)). This blood flow
that bypasses the capillary exchange network is carried in a
vessel called an arteriovenous anastomosis, or A-V shunt.

Studies of A-V shunts have in general been based on two
characteristic differences between the shunts and capillaries.
These differences are:

1) The thick wall of the A-V shunt vessel is
relatively impermeable to diffusion of metabolites and
ions when compared to the thin porous capillary wall.

2) The luminal diameter of the A-V shunt is considerably
larger than that of the true nutritive capillary.

Experimental studies exploiting the diffusional differences
between shunts and capillaries have used radioactive ions as
tracers for nutritional or capillary blood flow. These methods
have been concerned with the extraction of ions such as potassium
or rubidium, or inert gas such as xenon, from the blood to the
extravascular space following intraarterial introduction. In
addition the washout of ions such as sodium from the extra-
vascular space following intraarterial or intramuscular injection
has been used as an index of nutritional blood flow.

In our studies, we have studied A-V shunt flow on the basis
of the second characteristic difference mentioned above - namely
the larger luminal diameter of the A-V shunt compared to the
capillary. This was accomplished by the use of microspheres
(MSP) which are plastic spheres with a diameter too large to pass
through a capillary vessel, but small enough to pass all or most
of the A-V shunts. MSP are labelled with a gamma - emitting isotope such as $^{85}$Sr so that the quantitative accumulation of MSP at various tissue sites may be assayed with external radiation detectors.

What is the fate of a MSP introduced into a peripheral artery, and what physiological information can be deduced from this? In the peripheral vascular bed, the MSP will eventually encounter either a capillary or an A-V shunt. If a capillary is entered, the MSP will be locally lodged in that capillary. Therefore, with the assumption that MSP are valid tracers for blood flow, the peripheral accumulation of MSP is an indicator of capillary blood flow and the spatial distribution of peripherally trapped MSP corresponds to the spatial distribution of capillary blood flow. Using collimated radiation detectors, it is therefore possible to assay the relative capillary blood flow in various regions of tissue - subject of course to the limits of collimation, and the assumption that MSP trace flow.

Now consider a MSP that enters an A-V shunt. Since it is postulated that the A-V shunt lumen is larger than the MSP, the MSP will freely enter the venous return system, be carried to the right heart and then encounter the capillary bed of the lung. Our work indicates that, for diameters of 15 $\mu$m or larger, all MSP passing the peripheral bed are trapped in the lung. Thus the lung accumulation of MSP introduced into a peripheral artery is an indicator of shunt blood flow in the entire tissue supplied by that artery. If MSP are now introduced into a vein, all of them will be trapped in the lung, which would be equivalent to total or 100%
shunting with arterial introduction. Using the ratio then of lung trapping with venous and arterial introduction, the absolute shunt fraction in the peripheral bed is determined.

Prinzmetal in 1948 used non-radioactive glass spheres to show the presence of A-V shunts in different organs of several animal species. This work was strictly qualitative. Recovery of spheres from the venous side of the organ under study was taken as evidence of A-V shunts existing in that organ. Since that time spheres of several materials, usually with radioactive labels, have been used in studying A-V shunts with a progression towards more quantitative measurements. The commercial availability since 1967 of radioisotope labelled plastic MSP with tight size distributions has stimulated A-V shunt studies.

Perhaps one of the more important developments of our work has been the arterial introduction of MSP's as a constant-rate infusion, instead of as an injection. One advantage of the infusion method is in minimizing what Burton (1954) called "Reactive Error in Physiology" - namely that a physiologic system reacts to the stimulus of a measurement performed on it. This is analogous to the Heisenberg Principle of physical science, but cannot be as rigorously defined in physiology as in physics. Although we may not be able to quantitate our "reactive error" or "uncertainty principle" in physiology, we still must be cautiously aware of its existence and possible consequences. An infusion may cause some reactive error. The rate of MSP introduction with infusion is in general less than for injection. This could be expected to produce less perturbation on the system under study. More importantly
the stimulus of the infusion remains constant in time. Thus in
determining changes in a physiological system in response to other
stimuli, the experimental changes observed are more likely to be
related to the stimulus being studied than to an artifact of
the measurement. A further advantage of the infusion method is
that the system under study is being monitored continuously with
respect to time. This allows measurement of the kinetic response
of the system to physiologic manipulations as well as the intrinsic
time variation, i.e. the kinetics of the "normal" state.

I.B. Organization and Scope

In this presentation, we will first discuss some of the
general anatomic and physiological characteristics of peripheral
circulation. Specific responses of the circulation to exercise
and temperature will then be reviewed since these are the most
common stimuli encountered in the natural state.

Our MSP experiments included studies on the effect of
epinephrine on blood flow. Therefore a brief discussion on the
circulatory effects of this agent is given.

Next we present a review of circulation studies performed
with radioactive tracers and with MSP's. Some of the limitations
and problems associated with these methods will be considered.

Then follows a description of the experimental procedures
and the results obtained. These results include information on
the time variation of shunt flow, the effects of MSP size, the
effects of epinephrine, and the relative importance of shunt flow
in muscle and skin.
II. Physiology of peripheral circulation

II-A. General characteristics of peripheral blood flow


The aorta and its main branches are primarily elastic in function and contain relatively little muscle. The muscle that is present affects primarily the tensile properties of the arterial wall and does not cause active contraction. As these arteries divide, they become progressively smaller in diameter and richer in smooth muscle content. The latter factor allows contraction or dilatation of the vessel caliber.

The arterioles are primarily smooth muscle and account for most of the pressure drop in the circulatory system. Before the arterioles divide into capillaries there is a muscular ring of cells called the pre-capillary sphincter. This sphincter can periodically open and close, resulting in distal changes in capillary flow.

Capillaries are the exchange vessels, consisting of an endothelial cell monolayer surrounded by a basement membrane. The capillaries have no intrinsic ability to alter their diameter; however, the exchange function of these vessels can be affected by hydrostatic forces, plasma oncotic pressure, or agents such as histamine that influence permeability.
Venules collect blood from the capillary networks and merge into veins. Veins, especially in the lower extremities, have one-way flow valves and muscular walls to assist in returning blood to the heart. In addition to capillaries there are two other types of connections between arteriole and venule. These are the "thoroughfare channel" and the arterio-venous anastomosis. Both of these vessels have muscled walls and hence have little exchange function. They differ in that true capillaries branch off of a thoroughfare channel, while A-V anastomoses are a direct arteriovenous connection. A representation of the vessels is shown in Fig. 1.

Of primary interest to this thesis is the "microcirculation" which is concerned with blood flow in the arterioles, capillaries and venules. The arrangement of arterioles consists of a complex "arcade" system with numerous interconnecting channels or anastomoses. Capillaries and venules also exhibit this arcade pattern. These arcuate patterns, together with the ability of sphincters to intermittently open and close, leads to the striking observation of stasis or even retrograde blood flow in the capillary and venule networks.

The active vasomotion of arterioles is an important factor regulating flow and pressure in the capillary beds. Another possible control factor is flow through arterio-venous anastomoses. Since according to Poiseuille's equation, fluid flow is proportional to the 4th power of the tube radius, a few A-V anastomoses of 100 micron (\(\mu\)) diameter could carry significant flow compared to many 10 \(\mu\) diameter capillaries.
Fig. 1. Exchange routes between artery and vein (after Saunders et al. (1957)).
Skin blood flow has several functions in addition to cutaneous nutrition. The regulation of body core temperature and protection of tissue from freezing during extreme cold exposure are generally accepted roles. Other functions that have been suggested include a reservoir of blood for rapid supply of previously inactive tissue and the partial control of systemic blood pressure. In most of these roles, blood flow through arteriovenous anastomoses is an important factor. Skin blood flow in the human hand may vary from 1 to 100 ml/min/100 gm skin in response to temperature regulation requirements. The lower figure corresponds to the flow required for nutrition. Assuming that the blood in the skin always comes to thermal equilibrium with its surrounding tissue, heat transfer is proportional to flow. Hence this wide variation of flow results in regulatory ability over a wide temperature range. Much of the increased flow is through shunt vessels.

Regulation of flow through A-V shunts is at least partially under sympathetic nervous control. Sympathetic denervation increases skin flow by loss of vasoconstrictive tone, although this increase disappears after a few weeks. Chemical control also exists; epinephrine is a constrictor, bradykinin a dilator.

Blood flow in muscle is almost completely concerned with nutrition, although the presence of some A-V shunts has been claimed. Both arteries and arterioles in muscle form complex anastomosing arcades - a structure useful for establishing collateral circulation in response to trauma. Resting skeletal muscle blood flow is estimated to be 2 to 10 ml/min/100 gm while during exercise flow may increase to 40 ml/min/100 gm.
Arteries supplying muscle branch freely with frequent anastomoses to form a "primary network". Within the meshes of this net arises the secondary arteriolar network of vessels, again showing an arcade pattern. Capillaries in general run parallel to the muscle fibers, but with frequent cross connections so that the fibers are surrounded by a fine capillary mesh.

Muscle blood flow is controlled by local metabolic, central nervous, and humoral factors. Increased oxygen consumption in active muscle is accomplished both by increased unloading of hemoglobin and an increase in blood flow. Nervous control is demonstrated by increased blood flow in the sympathectomized limb, and increased blood flow in response to mental stress, or the anticipation of exercise. Epinephrine, nor-epinephrine, acetylcholine and histamine are some agents that effect humoral control of muscle blood flow.

II.B. Response to exercise

Several interrelated factors occur during exercise that influence muscle blood flow. Among these are autonomic nervous control, mechanical compression of vessels, elevated local heating, increased cardiac output and systemic blood pressure, and metabolic changes resulting in high carbon dioxide and lactic acid and low oxygen concentrations. In addition, vasodilator substances have been implicated by some authors as being important in blood flow response to exercise. Exercise does increase muscle blood flow; at the present time the exact mechanism of this effect is not understood.
Tetanic contraction causes first a brief flow increase due to compression of the filled vessels followed by a drop in flow rate due to occlusion of the arterial supply. Within a few seconds however blood flow increases to several times its resting value. This is most likely due to local, chemically mediated factors. After relaxation, blood flow increases slightly more and then returns to the resting level (Friedman and Selkurt, 1966).

Rhythmic contraction of muscle produces rhythmic variations in muscle blood flow. The alternate contraction and relaxation of the muscle produces, in the venous outflow, first an increase as blood is squeezed out of the tissue and subsequently a flow decrease as the vessels refill during relaxation. Superimposed on this pulsatile response is an overall hyperemia believed to be due to local metabolic vasodilators.

Muscle blood flow during exercise is proportional to the oxygen consumption of the muscle tissue. Rosell and Uvnas (1962) described some factors of nervous control of flow and oxygen uptake. Hypothalamic stimulation of vasodilator nerves produced an increase in blood flow and a decrease in oxygen uptake. Inhibition of vasoconstrictor tone also increased blood flow but now increased the oxygen uptake. One possible explanation proposed was that the adrenergic vasoconstrictor nerves were concerned with capillary flow while cholinergic vasodilator nerves influenced non-nutritional flow, perhaps through A-V anastomoses.

Barger et al. (1956) measured cardiac output, oxygen consumption rate and systemic arterial and venous oxygen concentrations in dogs during tread-mill exercise. Cardiac output increased linearly with oxygen consumption, but the A-V oxygen difference
was hyperbolically related to $O_2$ consumption.

These authors suggested that such relations were consistent with the hypothesis that A-V oxygen difference in muscle capillary flow remains constant and that the increased cardiac output is entirely directed to muscle tissue. Further experiments measuring $O_2$ in venous blood draining muscle specifically, rather than in mixed venous blood, showed that mild exercise increased the A-V oxygen difference from about 5 vol. % to about 13 vol. %. This result was explained on the hypotheses of parallel shunt and nutritive circulation in muscle and that at rest the non-exchanging shunts carry a large fraction of flow while exercise increases both the total flow and the fractional flow to nutritive capillaries.

II.C. Response to temperature

Temperature responses of the peripheral circulation occur almost entirely in the skin, primarily under hypothalamic control. It is well documented that arterial-venous anastomoses (AVA) play a large role in skin blood flow (Greenfield (1962)). This is especially true in the extremities and ears, which have high surface to volume ratios and hence are effective in thermoregulation. The AVA are vessels directly connecting arterioles and venules. They have thick muscular walls with an average lumen diameter of 35 μ. Grant and Bland (1931) reported 500-600 anastomoses per square centimeter in the human finger and toe nail bed.

When the skin is cooled, heat loss is reduced by two means. First, there is vasoconstriction of the skin vessels - primarily
the AVA. This in effect insulates the blood from the cold environment. Secondly the venous return blood is shifted away from the surface veins to more central vessels. Here the cool venous blood is warmed by countercurrent heat exchange with the arterial blood. Since this exchange of heat occurs locally in the extremity, it is an effective means of defending the central core temperature.

In studies of immersion hypothermia in dogs, Covino, et al. (1956) observed an increase in femoral arterial flow and a decrease in arterial pressure. The flow increased with time initially and later slowly decreased. The minimum A-V oxygen difference occurred when flow was maximum. The authors argue that the skin cools quickly and is at a circulatory equilibrium state while total limb flow is still increasing. Hence the delayed flow increase could be due to opening of AVA in the muscle.

We believe that this argument is questionable. Henshaw, Underwood and Casey (1971) performed temperature measurements on the foot pads of arctic wolves during immersion at -38°C. In several cases, pad temperature dropped rapidly to near 0°C, then abruptly rose to 15°C for 40 minutes and then fell to near 0°C again. This indicates that cutaneous circulation is not at equilibrium for some considerable time after immersion and hence the argument of Covino may be invalid.

Edholm, Fox and Macpherson (1956) studied the effects of whole body heating on forearm blood flow in man. Skin flow in one arm was occluded by iontophoresis of epinephrine. Blood flow in the untreated arm increased linearly with body temperature while the treated arm flow remained constant. They concluded that changes in skin flow were mainly responsible for the observed arm flow changes.
II.D. Response to epinephrine

Since the experimental work to be described later uses venous epinephrine infusion to alter blood flow, this section will describe some of the known actions of this agent on the cardiovascular system.

Epinephrine affects both cardiac output and the peripheral resistance to blood flow. The partial independence of these actions, together with a complicated dose-response curve, produce various responses dependent both on the route of administration (arterial or venous) and the level of dosage.

Abramson (1967) states that epinephrine increases cardiac output and that the systolic pressure rise is primarily due to direct cardiac action rather than a peripheral effect. Thus it follows that venous and arterial introduction will have different results. Arterial introduction will have a large local effect in the arterial bed under study, but simple dilution of the epinephrine with venous blood from the rest of the body will diminish the direct cardiac effect.

The peripheral effects of epinephrine involve the so-called alpha and beta receptors. In general α receptors are vasoconstrictive and β receptors are dilators. Innes and Nickerson (1970) describe the following scheme of action. Skeletal muscle vessels have both α and β receptors while skin vessels have only α. The β receptors are more sensitive to epinephrine and hence low doses produce only a β (dilator) effect and reduce blood pressure. Higher doses activate the α (constrictor) receptors and thus increase peripheral resistance. Since cardiac output is increased by epinephrine also, the net effect is a decrease in skin blood flow and increase in muscle flow.
III. Nutritional blood flow measured with diffusable ions

III.A. Washout methods

The study of regional blood flow with diffusible radioactive ions probably originates with the work of Kety (1948). Basically the concept is as follows. Consider the extravascular space in a region of interest labelled with an ion such as Na\(^{24}\). This tracer ion diffuses through the capillary wall and into the blood stream and hence is cleared from the local area. If there exists no diffusion limitation of ion transport at the blood flow rates encountered, then the rate of clearance is proportional to flow and we may write

\[ \dot{C} = - \frac{1}{k} \cdot F \cdot C \]  

(1)

where \( C \) is the ion concentration, \( k \) is a proportionality factor and \( F \) is the flow. Assuming \( F \) to be constant with time, we have

\[ C(t) = C(0) \cdot e^{-Ft/k} \]  

(2)

Now \( k \) is seen to have dimensions of volume. It should be emphasized that \( k \) represents a virtual volume and does not necessarily correspond to a physiologic region.

The above development, written as

\[ C(t)/C(0) = e^{-Ft/V} \]  

(2)

is the result of "compartmental" analysis. The extension of this single compartment model to one consisting of three parallel compartments is discussed by Dobson and Warner (1957).

A more generalized treatment of this problem, involving the concept of mean transit time has been discussed by Meier and Zierler (1954) and Bassingthwaight (1970). The result of this development is that the mean transit time, \( \bar{t} \), for an ion to traverse a system is
\[ \bar{\tau} = \frac{V}{F} \]  
(4)

where \( V \) is a virtual volume and \( F \) is the flow through the system. It is shown that using an external collimated radiation detector and an instantaneous delta function introduction of tracer, the mean transit time is found by

\[ \bar{\tau} = \frac{\int_{0}^{\infty} R(t) \, dt}{R(0)} = \frac{V}{F} \]  
(5)

where \( R(t) \) is the detector count rate. This analysis has the advantage that it is model independent, and is particularly useful with isolated preparations where \( F \) may be measured directly.

Using the proportionality between local concentrations and count rates, it is of course seen that the result from equation 3 is a special case of equation 5. Let

\[ R(t) = a \cdot C(t) \]  
(6)

and equation 5 becomes

\[ \bar{\tau} = \frac{a \cdot \int_{0}^{\infty} C(t) \, dt}{a \cdot C(0)} \]  
(7)

and substituting equation 3

\[ \bar{\tau} = \frac{a \cdot C(0) \int_{0}^{\infty} e^{-\frac{F}{V} t} \, dt}{a \cdot C(0)} \]

\[ = \frac{V}{F} \]  
(8)

The concept of capillary and shunt flow in muscle existing as parallel components has been studied by Renkin (1971) and Barlow, Haigh and Walder (1961). Renkin used washout of antipyrine to measure blood flow in the isolated perfused hindlimb of the cat. He found that nutritive flow was about 80% of total flow in the intact limb, but that essentially all flow was nutritive when skin and paw were removed, indicating no shunt flow in muscle.
Barlow et al. used both intact animals and semi-isolated preparations in their studies of Na\textsuperscript{24} washout. They injected Na\textsuperscript{24} intramuscularly and attempted to influence the clearance rate with intravenous epinephrine. The results were quite erratic which possibly could be due to the intramuscular injection, a problem that had previously been studied by Warner, et al. (1953). With intra-arterial injection, however, Barlow found that the clearance curve could be fitted by two exponential components. Epinephrine increased the clearance rate of the faster component and had no effect on the slower component. On the basis of small localized injections, the fast component is said to be associated with muscle fibers while the second is attributed to intramuscular septa and tendons. These findings support the concept of parallel capillary and shunt blood flow in muscle, but the relative magnitudes are not stated.

The use of ion washout curves in the clinical evaluation of intermittent claudication (leg cramping following exertion) has favored the use of \textsuperscript{133}Xe as opposed to \textsuperscript{24}Na. This is because of the better diffusional properties of xenon which make it a better index of blood flow when using the clinically preferred intramuscular administration. Lassen (1964) directly compared clearance of \textsuperscript{24}Na and \textsuperscript{133}Xe in both normal and diseased subjects. The results indicate good agreement at resting flow rates but \textsuperscript{24}Na clearance suggested diffusion limitation during reactive hyperemia. Thus it is concluded that \textsuperscript{133}Xe is preferable for clinical evaluation of circulatory status. Alpert, del Rio and Lassen (1966) report on the effects of treadmill exercise on \textsuperscript{133}Xe clearance. While washout in the resting state was similar in normals and patients with intermittent claudication, the response to exercise was markedly
different. Patients showed a smaller increase in clearance rate during exercise than normals and a slower return to preexercise levels. Both results are consistent with impaired nutritional blood flow in the patient group and the authors recommend this test as a diagnostic tool in evaluating circulatory status.

III.B Extraction methods

The previous developments are concerned with the removal of an extravascular tracer by the blood. Another method of regional blood flow study utilizes the deposit of radiotracer in extravascular regions to assess volumes and blood flows, as reviewed by Sapirstein (1967). Ions such as potassium and rubidium wash out of the intracellular space very slowly. This fact favors the use of these ions for measurement of cardiac output distribution. The problem arises from wash out of tracer from the extracellular fluid during the time from administration to sacrifice of the animal. To overcome this, several animals are injected and serially sacrificed, followed by back extrapolation to injection time to arrive at an organ flow figure. Ions with a slow wash out component thus make the extrapolation less risky.

These extraction methods have been used in the study of nutritive blood flow. Friedman (1971), (1965) reported on rubidium extraction in the isolated dog gracilis muscle. He concludes that total muscle flow is greater than capillary blood flow and that capillary permeability is non-limiting in rubidium extraction. This would imply the existence of non-nutritive shunt vessels in muscle.
IV. Microsphere studies of blood flow

Some of the early circulation studies with microspheres were done by Prinzmetal and collaborators using glass MSP. [Prinzmetal et al., (1947), (1948), Simkin et al. (1948)]. These qualitative studies concerned heart, lung, kidney, liver and spleen in both humans and animals. It was concluded that arterio-venous anastomosis (AVA) were generally present in most tissues. Mendlowitz (1957) described the medical importance of AVA in various pathological states.

The near universality of AVA has been questioned. Knisely et al. (1956) criticized Prinzmetal's results, especially in regard to lung shunts, on the basis of retrograde blood flow. Prinzmetal claimed the existence of lung shunts on the basis of MSP recovery in the liver following intravenous injection. Knisely repeated this experiment with the inferior vena cava prepared so as to prevent retrograde entry to the liver. No spheres were found in the liver by Knisely. Another criticism of MSP studies has been the high density of glass spheres—possibly yielding incorrect results due to settling of the spheres in the blood stream. For this reason, MSP of polystyrene, or human serum albumin [Rhodes et al. (1969)] have been used more recently.

Rudolph and Heymann (1967) measured organ blood flow in the fetus of sheep and goats using 50 μ MSP. In both in vitro and in vivo studies, they compared MSP distribution with direct flow measurements and concluded that MSP
are valid tracers of flow, at least in large diameter vessels. Since flow distribution to organs is determined primarily in large vessels, they further conclude that organ MSP accumulation is proportional to organ blood flow. Similarly, Forsyth et al. (1968) injected MSP into the left heart to determine the distribution of cardiac output in the rhesus monkey. Other
representative studies of regional blood flow include Delaney (1969) on the mesentery, Blum et al. (1970) on the heart, and Valencak et al. (1971) on the brain.

The validity of MSP measurements of cardiac output distribution is considered by Hoffbrand and Forsyth (1969). They conclude MSP's are well mixed with arterial blood at least down to the femoral artery. Additionally, concentrations of MSP's in blood from the femoral and renal arteries were equal, implying that the MSP's show no preferential distribution at this level of the circulatory tree.

Phibbs and Dong (1970) made direct observations on MSP mixing in the rabbit's femoral artery, using special techniques to freeze the artery in less than 50 msec. MSP diameters from 7.5 to 80 μ were tested by sectioning the frozen artery and microscopically determining the radial distribution of MSP locations. They found that in general there was a preferential axial accumulation of MSP and that the departure from uniformity increased for larger values of MSP diameter to luminal diameter ratio. On this basis, these authors suggest that MSP are valid tracers for total organ blood flow since only large arteries would be involved, but that more microscopic determinations of flow distribution could be influenced by the observed nonuniformity. We will discuss the effect of this possibility on our results in a later section.

MSP studies of the peripheral circulation in the extremities have mainly been performed by Wagner and collaborators [Spence, Rhodes and Wagner (1972), (1969), Rhodes et al.
Radioactive MSP's are injected into a peripheral artery, and an external radiation detector is placed over the lung. The fraction of injected dose reaching the lung is hypothesized to represent the fraction of blood flow through AVA. The counting efficiency of the external detector is established by an intravenous microsphere injection which corresponds to total shunting. Thus absolute percent shunt may be determined.

At this point, let us examine the consequences of partial bypass of MSP through the lung on the measurement described above. Consider the following: let

- \( N_0 \) = number of MSP introduced in a peripheral artery
- \( N_T \) = number of MSP trapped in lung
- \( f_P \) = bypass fraction for the peripheral bed
- \( f_L \) = bypass fraction for the lung
- \( f_S \) = bypass fraction for the total circulation

Then on the first lung passage, \( N_0 \cdot f_P \cdot (1 - f_L) \) is the number of MSP trapped while \( N_0 \cdot f_P \cdot f_L \) MSP pass through the lung. On each subsequent lung passage, the number of MSP trapped is reduced by the factor \( f_S \cdot f_L \) so that the total lung trapping given by

\[
N_T = N_0 \cdot f_P \cdot (1 - f_L) + N_0 \cdot f_P \cdot (1 - f_L) \cdot f_S \cdot f_L + N_0 \cdot f_P \cdot (1 - f_L) \cdot f_S^2 \cdot f_L^2 + \cdots
\]

\[
= N_0 \cdot f_P \cdot (1 - f_L) \cdot \sum_{n=0}^{\infty} (f_S \cdot f_L)^n
\]

\[
= N_0 \cdot f_P \cdot (1 - f_L) \cdot \frac{1}{1 - f_S \cdot f_L}
\]

A similar development for \( N_T' \), the number of MSP trapped in the lung for venous introduction of \( N_0 \) MSP, yields
\[ N_T' = N_0 \cdot (1-f_L) \cdot \frac{1}{1-f_S f_L} \]

Thus the measured bypass fraction \( N_T/N_T' \) is equal to \( f_p \) only if both \( f_S \) and \( f_L \) do not change during the time between arterial and venous introduction. For the special case where \( f_L \) is zero for all times, the value of \( f_S \) is immaterial and the ratio \( N_T/N_T' \) is a valid measure of \( f_p \).

This result applies to MSP introduction by means of injection. For a MSP infusion, the same sort of result is obtained.

Let

\[ I = \text{MSP infusion rate} \]
\[ R = \text{MSP accumulation rate in the lung} \]

Then it can be shown that \( R \) approaches an equilibrium value

\[ R_{eq} = I \cdot f_p \cdot (1-f_L) \cdot \frac{1}{1-f_S f_L} \]

for arterial infusion and

\[ R_{eq}' = I \cdot (1-f_L) \cdot \frac{1}{1-f_S f_L} \]

for venous infusion. Again, as for an injection, taking ratios of arterial to venous accumulation rates gives a correct result for the bypass fraction providing that \( f_L \) and \( f_S \) are constant, or that \( f_L \) is zero.

Although some authors report shunting of 75 to 500 \( \mu \) diameter spheres through the lung [Parker, Anderson and Smith (1958); Rahn, Stroud and Tobin (1952); Tobin and Zariquiy (1950)], Ring et al. (1961) reported no spheres larger than 15 \( \mu \) would pass the pulmonary capillaries. It is most likely that Ring's results are correct since the other studies involved either denervated heart-lung preparations or arterial irritation by a catheter - conditions likely to induce unnatural shunting.
V. Materials and methods
V-A. Animal preparation

Adult beagle dogs weighing from 9 to 13 kg were used in these experiments. The anesthesia procedure was started by intravenous injection of sodium thiopental (Surital\textsuperscript{R}, Parke-Davis) to effect, usually 0.5 cc/kg of 4% solution. Then an endotracheal tube was introduced and connected to a Heidbrink veterinary anesthesia machine (Ohio Medical Products). Anesthesia was maintained with methoxyflurane (Metofane\textsuperscript{R}, Pitman-Moore). In experiment number 14, sodium pentobarbital (Diabutal\textsuperscript{R}, Diamond Labs) was used throughout with an initial dose of 30 mg/kg and infusion maintenance of 1 mg/min.

Immediately after induction of anesthesia, body temperature of the dog was stabilized by using heating pads controlled by a proportional temperature controller (Yellow Springs Instruments Model 72) and a thermocouple in the esophagus. Arterial blood pressure recordings were obtained with a pressure transducer (Statham Labs, model P23Db) and a recording oscillograph (Visicorder\textsuperscript{R}, Honeywell Denver Division, model 1108).

The jugular and saphenous veins were catheterized with PE 50 tubing for infusion of epinephrine and MSP respectively. Next a 22 gage hypodermic needle with hub removed was attached to PE 50 tubing and used for percutaneous puncture of the femoral artery in the region of the femoral triangle. The needle was inserted in retrograde direction to promote good mixing of MSP with the arterial blood.
After completion of the MSP infusions, the dog was usually examined on a multi-detector whole body scanner (Anger (1972)). This scan indicated whether any significant extravascular infusion had occurred and verified that the MSP were located in lung and leg only. Figure 2 shows scans from two experiments. In the top picture, where both legs were infused, it is obvious that the left leg infusion was partially extravascular. This experiment was rejected on that basis. The lower scan indicates a good infusion. Activity is confined to the lung and leg, and no "hot spot" is observed at the infusion site.

V-B. Microsphere characteristics

Carbonized microspheres (3 M Nuclear Products) of nominal 15 or 35 micron diameters were used for all experiments. The MSP were labelled with either $^{85}\text{Sr}$ or $^{141}\text{Ce}$ to permit external monitoring of the associated gamma radiation. Size distributions of the MSP were measured directly using a Zeiss Ultraphot II microscope with calibrated eyepiece reticle. Results indicated that the MSP diameters were quite near the nominal value. A typical result for a "15 micron" batch was a mean diameter of $17.8 \pm 0.3 \mu\text{m}$ with a population standard deviation of $2.2 \mu\text{m}$. Photomicrographs of this lot are shown in Fig. 3 at low and high magnification. The low magnification indicates some tendency for spheres to "pair" but no large clumping is observed. The concentration of MSP observed here approximates that used in the infusion fluid. The higher magnification shows the smooth, highly spherical shape of the MSP.
Fig. 2. Scanner results for a partial extravascular infusion (above) and for a valid infusion (below). Both legs were infused in the upper picture.
Fig. 3. Photomicrographs of nominal 15 micron MSP.
V-B-1 Infusion technique

The constant rate infusion of MSP, despite their tendency to settle, was accomplished by the use of a special infusion pump originally designed for studies involving colloidal chromic phosphate. This pump has a rotating bar magnet located under the infusion syringe. The magnet also oscillates horizontally for the length of the syringe so that glass coated magnetic stirring rods inside the syringe agitate the entire internal volume. This pump was modified slightly to accept a standard 50 cc disposable syringe rather than the lucite syringe originally used. A photograph of the infusion pump is shown in Fig. 4.

MSP as received from the supplier were diluted to the desired specific activity with 6% dextran 70 (Cutter Labs) to which had been added 0.05% sorbitan mono-oleate (Tween 80, Fisher Scientific) and 4 units/cc heparin (heparin sodium, Upjohn). Dextran was used to reduce MSP settling rate, Tween 80 to lessen clumping of MSP, and heparin to prevent blood clotting at the needle tip. The fluid infusion rate was 0.17 cc/min.

The infusion rate of MSP radioactivity was typically 0.5 μCi/min. As received, MSP had a nominal specific activity of 10 μCi/mg which corresponds to infusion of 0.05 mg/min or about 20,000 MSP/min of 15 μ diameter. Does infusion of this number of MSP in itself alter blood flow due to blockage of a significant number of capillaries? A study by Rhodes et al. (1972) indicates that it does not. Using 15-30 μ diameter albumin MSP, they found that injected doses on the order of 10 mg MSP/kg perfused tissue were necessary to produce blood flow disturbances. Since a hind
Fig. 4. Magnetically stirred infusion pump. (Original design by Dr. L. Finkelstein and Mr. P. Dowling)
leg is about 0.7 kg, infusion for over 100 minutes would be necessary to produce noticeable effects due to capillary blockage. No infusion was made for this length of time.

In the experiments using epinephrine infusion, epinephrine (Adrenalin \textsuperscript{R} chloride, Parke-Davis) was diluted to the desired concentration with normal saline shortly before administration. The dosage was 1 \( \mu \text{g} \text{m}/\text{min}/\text{kg} \) body weight for all experiments except numbers 1 and 2 where the infusion was 2 \( \mu \text{g} \text{m}/\text{min}/\text{kg} \). Typical response of arterial blood pressure to epinephrine infusion is shown in Fig. 5.

V-C. Data collection

The radiation detectors used were NaI (Tl) scintillation crystals, 1\( \frac{1}{4} \)" diameter by 1" thick, with straight bore lead collimators 2" diameter by 1-3/4" long. Conventional amplifiers and single channel pulse height analysers were interfaced to a PDP-12 (Digital Equipment Co.) computer with 8K memory and a 12 bit word length. Up to 8 data lines could be handled simultaneously, thus allowing for instance the use of 4 detectors and 2 different isotopes.

The assembly-language program for data accumulation was written by Mr. Robert Belshe of the Lawrence Berkeley Laboratory computations group. In outline, this program records the number of events occurring in a certain time interval (epoch) in buffer memory locations. At the end of an epoch, count storage is shifted to a second buffer array and the data from the previous epoch transferred to the memory storage area, along with the epoch duration. In subsequent epochs, current data storage alternates
Fig. 5. Blood pressure response to intravenous epinephrine (1 μg/min/kg)
between the two buffer arrays. One-half the memory (4096 locations) is used for storage. Thus if 4 count channels and a time channel are used, over 800 epochs may be stored in one run.

During an experiment the computer's oscilloscope display presents dynamically the count rate vs. time curves collected, and several informational and control parameters. This display is shown in Fig. 6. The left column of figures are count rates for input channels 1-8 during the last completed epoch. The other two columns show experimental parameters. These include:

TL = time limit, the epoch length in "ticks", 50 ticks = 1 second
RL = run length, in minutes
RN = run number
CE = maximum number of counts recorded in previous epoch
ET = elapsed time of run
T = time of day

The count rate curve(s) to be displayed are operator controlled by keyboard sense switches.

At completion of a run the stored data is transferred to magnetic tape for later analysis, which is the subject of the next section.

V-D. Data analysis

At the completion of an experiment, the accumulated data consists of epoch lengths and number of counts recorded as binary numbers on magnetic tape. The object of analysis is to obtain slopes of the count rate vs. time curves according to a linear least
Fig. 6. Computer display during operation of data collection program.
squares fitting criterion. This analysis was performed on the
PDP-12 computer using programs that we have written, in the
FOCAL-12 language. Program listings are presented in
Appendix II.

Analysis on the small computer, rather than on a large
machine with FORTRAN, was preferred because the FOCAL-12 programs
allowed operator interaction and decisions during the data pro-
cessing. Operations such as correction of obviously erroneous
data points (dropped channels) or changes in time interval for
linear fitting were easily accomplished in the FOCAL-12 analysis.
With large machine processing, each such change would require a
one day (or longer) turnaround time.

The programs used were called START, DPL0T, LINE, LINE 1
and, for the two isotope experiments, XTALK. Program START takes
the integer recorded data and transfers it to another tape in
floating point format. Epoch lengths in ticks are converted to
minutes. Program DPL0T then produces a visual display of count
rate vs. time data for a chosen channel. Scaling of the curve is
done within the program. The full scale count rate and time are
presented, along with curve identification and time scale ticks each
two minutes. A photograph of the display is taken for future
reference (see Fig. 8, p. 45, for an example).

Next the programs LINE and LINE 1 are used to find curve
slopes over an operator chosen time interval. The time interval
is chosen on the basis of the display from DPL0T; the validity of
this choice is checked by a plot of the data point residuals where
non-linearity of the data would produce a patterned rather than
random display. Output data includes the slope, its standard deviation, the intercept and the goodness of fit parameter (COF, Appendix I).

An additional step was necessary in the experiments with two sizes of MSP, one labelled with $^{85}$Sr and one with $^{141}$Ce. Prior to starting the infusions, a small number of the higher energy $^{85}$Sr MSP were injected arterially. This allowed calibration of each external counter for scattered radiation in the $^{141}$Ce channel due to $^{85}$Sr. In the mix infusion, the $^{141}$Ce specific activity was made large enough relative to $^{85}$Sr that scatter corrections of only 10-20% were necessary. Given the scatter or cross-talk factors determined from the $^{85}$Sr MSP injection, the program XTALE accomplished the necessary correction of the counts in the $^{141}$Ce channel during mix infusion.

After slopes had been obtained, the overall leg shunt fraction was found as the ratio of lung slopes for arterial infusion and venous infusion. This ratio gives the leg shunt fraction since the venous infusion bypasses the leg microcirculation and produces a count rate slope corresponding to 100% shunt flow. With arterial infusion some MSP are locally trapped resulting in a lung curve slope which is proportional to the number of MSP bypassing the leg. Since the rate of infusion is equal in the arterial and venous cases, the ratio of slopes for arterial and venous infusions gives the absolute leg shunt flow fraction, as measured by leg passage of MSP.

For the purpose of comparing results from different experiments on the effect of epinephrine (e.g. Table 3) slopes during
and post epinephrine are expressed relative to the pre-epinephrine slope. In this manner inter-experiment differences in infusion rate of activity and radiation detector efficiency are cancelled out.
VI. Results and discussion

VI.A. Magnitude of shunt flow and its constancy with time

A total of 22 experiments were performed; results from 6 experiments were rejected because of evidence of extravascular infusion from the whole body scan or because of obvious misfunction of the infusion pump or electronics system. The remaining 16 experiments were accepted as valid. In 14 experiments the shunting fraction for 15 micron MSP's was determined in the normal anesthetized state. The shunt fractions, usually measured with two independent lung detectors, are presented in Table 1 along with the goodness of fit parameter (G.O.F.). As detailed in Appendix I, a G.O.F. near unity indicates good data fit to a linear hypothesis.

The shunt fractions observed had a range from 33% to 93% and a mean value of 62%. These results are reasonably in agreement with other studies. Spence, Rhodes and Wagner (1972) using 20 micron albumin MSP's report shunt fractions ranging from 0% to 88%, the average being 20%. With 36 micron albumin MSP's, Lopez-Majano, Rhodes and Wagner (1969) observed shunt fractions ranging from 1% to 59%. A possible point of disagreement is that we observe no shunt fraction under 30% while both of the above reports indicate many results in the 0% to 10% range. One possible explanation is that purebred beagle dogs were used in our work while mongrels were used in other studies. It is certainly conceivable that there are species differences in peripheral shunting and, if so, a purebred line would exhibit less variation than mongrels. Another possibility is that our use of body temperature regulation resulted in
Table 1. Shunt fractions of 15 micron MSP in the normal anesthe-
tized dog

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>% Shunt Detector 1</th>
<th>% Shunt Detector 2</th>
<th>Average of both detectors</th>
<th>Goodness of fit Detector 1</th>
<th>Goodness of fit Detector 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>71.6 ± 0.9</td>
<td>64.2 ± 1.6</td>
<td>67.9</td>
<td>0.89</td>
<td>1.23</td>
</tr>
<tr>
<td>2</td>
<td>34.3 ± 0.7</td>
<td>31.3 ± 0.7</td>
<td>32.8</td>
<td>1.21</td>
<td>1.10</td>
</tr>
<tr>
<td>3</td>
<td>92.8 ± 2.7</td>
<td>94.0 ± 2.0</td>
<td>93.4</td>
<td>0.89</td>
<td>0.93</td>
</tr>
<tr>
<td>4</td>
<td>54.2 ± 2.8</td>
<td>52.8 ± 2.5</td>
<td>53.5</td>
<td>1.02</td>
<td>1.03</td>
</tr>
<tr>
<td>5</td>
<td>94.3 ± 3.5</td>
<td>86.8 ± 2.8</td>
<td>90.6</td>
<td>1.22</td>
<td>0.87</td>
</tr>
<tr>
<td>6</td>
<td>42.7 ± 4.7</td>
<td>34.0 ± 4.3</td>
<td>38.3</td>
<td>0.95</td>
<td>0.95</td>
</tr>
<tr>
<td>8</td>
<td>84.8 ± 3.7</td>
<td>81.1 ± 3.2</td>
<td>83.0</td>
<td>1.05</td>
<td>0.87</td>
</tr>
<tr>
<td>9</td>
<td>47.3 ± 3.1</td>
<td>55.5 ± 3.7</td>
<td>51.4</td>
<td>1.03</td>
<td>1.08</td>
</tr>
<tr>
<td>10</td>
<td>61.8 ± 3.7</td>
<td>—</td>
<td>61.8</td>
<td>0.96</td>
<td>—</td>
</tr>
<tr>
<td>11</td>
<td>65.4 ± 1.9</td>
<td>—</td>
<td>65.4</td>
<td>1.03</td>
<td>—</td>
</tr>
<tr>
<td>12</td>
<td>52.8 ± 1.9</td>
<td>—</td>
<td>52.8</td>
<td>0.91</td>
<td>—</td>
</tr>
<tr>
<td>13</td>
<td>66.9 ± 2.1</td>
<td>—</td>
<td>66.9</td>
<td>1.16</td>
<td>—</td>
</tr>
<tr>
<td>14</td>
<td>40.1 ± 1.3</td>
<td>42.2 ± 1.5</td>
<td>41.1</td>
<td>1.12</td>
<td>1.11</td>
</tr>
<tr>
<td>16</td>
<td>73.2 ± 2.3</td>
<td>79.0 ± 2.9</td>
<td>76.1</td>
<td>0.89</td>
<td>0.93</td>
</tr>
</tbody>
</table>

Mean = 62.5%  Mean of all

G.O.F. = 1.03
maintaining high skin shunt flow. Body cooling during anesthesia could perhaps result in lowered shunt flow.

Finally, the infusion itself could possibly result in increased shunting compared to the measurement made with injected MSP. This last explanation is however considered unlikely.

The possible influence of anesthesia on shunt flow was investigated in experiment 14 where sodium pentobarbital was used instead of the usual methoxyflurane. The observed 41% shunt was somewhat lower than average, but was not atypical. On the basis of this trial, there appears to be no large difference in shunt fraction due to these two anesthetics.

A significant result of our work is the unanimous finding that all G.O.F. numbers are close to unity. Thus we conclude that during the period of measurement (generally 8-12 min.) the total shunt flow through the leg is constant. This is in direct contradiction to the reports mentioned just previously, as well as that of Rhodes (1971). All of these reports state that the shunt fractions, as measured by serial arterial injections, are significantly variable in time—even over periods as short as 5 minutes. This disagreement may be an artifact, caused by the injection. Two causes of this effect can be suggested. First, the manual injection cannot be accomplished at an absolutely uniform rate. If uniform mixing of MSP's with arterial blood does not occur, then variable turbulence caused by differences in injection rate could influence the proportion of MSP's carried to shunt vessels. There is no direct evidence that the infusion of MSP's results in uniform mixing; the mixing during infusion however should at least be consistent. The second possible effect is that local pressure changes
caused by the injection and subsequent syringe flushing could influence the shunt flow. Since AVA have been postulated to partially regulate local blood pressure and flow, pressure changes at the arterial injection site could perhaps influence shunt flow in the distal microcirculation.

For our infusion experiments, counts were generally accumulated for 6 seconds per data point. The average leg shunt flow for these intervals was constant for periods of 8 to 12 minutes. In view of the active vasomotion reported for A-V shunts, (Liebow (1962)) this constancy of average shunt flow may seem surprising. Evidently the regulation is such that although individual vessels may change their flow characteristics, the average flow through many such vessels is remarkably constant in time.

VI-A-1 Comparison of infusion and injections

Experiments 10 and 11 were used to compare the shunt fraction as measured by an infusion and by serial injections of MSP. The results are summarized in Table 2. The count rate increases for lung, paw and thigh with arterial introduction are presented relative to the corresponding lung increases for venous introduction, i.e. count rate slope for infusion, or count rate increase for single injection.

In both experiments there is good agreement between shunt fraction, as measured in the lung, for the initial infusion and for the first injection. Subsequent measurements by injection, or by infusion in exp. 11, consistently result in a lower shunt fraction. This suggests that in some manner an injection alters the
Table 2. Comparison of infusions and injections of MSP

<table>
<thead>
<tr>
<th>Method of Arterial Introduction</th>
<th>EXP</th>
<th>Normalized* % Accumulation in:</th>
<th>Lung</th>
<th>Paw</th>
<th>Thigh</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>Infusion 1</td>
<td>61.8 ± 3.7</td>
<td>221 ± 12</td>
<td>33.1 ± 2.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Injection 1</td>
<td>58.5 ± 2.1</td>
<td>162 ± 4.4</td>
<td>60.3 ± 1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Injection 2</td>
<td>25.8 ± 2.3</td>
<td>269 ± 6.8</td>
<td>58.7 ± 1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Injection 3</td>
<td>18.7 ± 3.8</td>
<td>261 ± 10.0</td>
<td>76.5 ± 3.4</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>Infusion 1</td>
<td>65.4 ± 1.9</td>
<td>53.7 ± 1.6</td>
<td>25.3 ± 1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Injection 1</td>
<td>68.7 ± 3.6</td>
<td>51.7 ± 2.5</td>
<td>22.3 ± 1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Injection 2</td>
<td>65.7 ± 2.5</td>
<td>55.5 ± 2.0</td>
<td>24.7 ± 1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Injection 3</td>
<td>60.1 ± 2.6</td>
<td>58.6 ± 2.3</td>
<td>25.3 ± 1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Injection 4</td>
<td>57.0 ± 2.4</td>
<td>82.9 ± 3.9</td>
<td>24.9 ± 1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Injection 2</td>
<td>47.1 ± 2.6</td>
<td>98.0 ± 3.4</td>
<td>28.3 ± 1.6</td>
</tr>
</tbody>
</table>

* The normalizing factors used are the lung accumulations for venous introduction (slope of count rate curve for infusion or count rate increase for injection). With this normalization, the lung data shown are the absolute shunt fractions. The paw and thigh data are relative measures of local accumulation and have no absolute significance.
shunt flow characteristics in the leg, and that this alteration occurs not precisely at the time of injection, but shortly thereafter. Injections were made at intervals of 10-15 minutes.

The most drastic change in shunting occurred between injections 1 and 2 of exp. 10. This is interesting since while preparing for injection 2 we noticed that the indwelling arterial needle had become blocked. The needle and catheter were vigorously flushed with saline until free return flow of arterial blood indicated that the clot had been cleared. The subsequent large drop in shunt fraction supports the idea that pressure changes at the injection site may produce distal changes in shunt flow.

A decrease in lung accumulation (shunt fraction) is accompanied by an increased accumulation in either thigh, paw or both - as would be expected. Exp. 10 shows no consistent pattern of increase in thigh and paw. From injection one to injection two the paw increases while thigh remains essentially unchanged. With injections 2 and 3, the opposite happens - paw is constant and thigh increases. In exp. 11 the major increase in accumulation of MSP occurs in the paw while thigh accumulation changes only slightly, if at all.

We will now show that a simple model, as shown in Fig. 7, is not consistent with the above results. Assume the leg consists only of two tissues, thigh and paw. Also that the thigh and paw detectors see tissue that is representative of these two compartments, i.e. there is homogeneous response. Then the observed count rates for thigh, paw and lung are:
Fig. 7. Simplified model of microsphere accumulation.
where:

\[ C_i = \text{count rate over thigh, paw or lung} \]

\[ \epsilon_i = \text{corresponding detector efficiency factor} \]

\[ f_1 = \text{fraction of MSP introduced that enter thigh compartment} \]

\[ k_1 = \text{fraction of MSP entering thigh that are trapped} \]

\[ k_2 = \text{fraction of MSP entering paw that are trapped} \]

\[ I = \text{number of MSP injected} \]

Substituting (1) and (2) in (3):

\[ C_L = \epsilon_L \left[ \frac{1 - C_T}{\epsilon_T} - \frac{C_P}{\epsilon_P} \right] \]

Now for a venous injection, the lung count rate is \( \epsilon_L I \). Therefore dividing through by the venous injection count rate, and denoting the count ratios as \( C_i' \):

\[ C_L' = 1 - \frac{\epsilon_L}{\epsilon_T} C_T' - \frac{\epsilon_L}{\epsilon_P} C_P' \]

Since detector efficiencies are constant,

\[ 1 - C_L' = a C_T' + b C_P' \]

where \( a \) and \( b \) are constants.

An unweighted least squares fit was then used to determine \( a \) and \( b \) for experiments 10 and 11. Assuming all the lung accumulation to have a common standard deviation of 2.5%, a goodness of fit parameter (G.O.F., appendix 1) is found that has an expected value of one if the model is valid. We find G.O.F. values of 200 and 11
for exp. 10 and 11 respectively. Thus we reject the hypothesis that
the leg consists of thigh and paw compartments only, and/or that
these compartments react homogeneously throughout the leg.

VI-B. Response of shunt flow to epinephrine

It is well known that epinephrine has a strong vasostrictive
action of skin circulation. Since it is believed that much of the
peripheral shunt flow may be associated with skin, we decided to
investigate epinephrine infusion as a means of altering shunt flow.

Typically we found that the intravenous infusion of epinephrine
greatly decreased leg shunt flow as measured by lung accumu-
lation of MSP. Within the leg, muscle accumulation increased while
paw accumulation decreased. Typical counting rate curves obtained
before, during and after epinephrine infusion are shown as Fig. 8.
The results of 9 experiments involving epinephrine infusion are
presented in Table 3. The count rate curves during and after the
epinephrine infusion were reasonably linear. However goodness of
fit factors from 1.5 to 2 were common and we believe that this indi-
cates some departure from a completely steady state condition.

Some interesting patterns are apparent from the data in
Table 3. The ratio of lung slopes post epinephrine to pre-epinephrine
are consistently less than one, indicating a decreased shunting after
epinephrine that persists for over 20 minutes as a reasonably steady
state condition. The decreased shunting during epinephrine infusion
is in all cases accompanied by a decrease in paw accumulation (Slopes
during/pre < 1) and an increase in thigh accumulation (Slopes during/
pre > 1).
Fig. 8. Response of count rate curves to epinephrine infusion from 20-30 minutes.
Table 3. Effect of epinephrine infusion on 15 micron microsphere accumulation

<table>
<thead>
<tr>
<th>EXP</th>
<th>Lung</th>
<th>Paw slope ratios</th>
<th>Thigh slope ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Slope ratios:</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>During Epin.</td>
<td>Post Epin.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post Epin.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pre Epin.</td>
<td></td>
</tr>
<tr>
<td>Pre Epin.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Shunt %</td>
<td>During Epin.</td>
<td>Post Epin.</td>
</tr>
<tr>
<td>-------</td>
<td>--------</td>
<td>---------------</td>
<td>--------------</td>
</tr>
<tr>
<td>1</td>
<td>67.9±</td>
<td>-1.8±3.5</td>
<td>.68±.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>32.8</td>
<td>6.1±1.6</td>
<td>.51±.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>93.4</td>
<td>30.5±3.1</td>
<td>.83±.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>53.5</td>
<td>4.0±3.8</td>
<td>.86±.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>90.6</td>
<td>24.4±2.2</td>
<td>.71±.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>63.7</td>
<td>20.7±4.3</td>
<td>.69±.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>83.0</td>
<td>19.6±3.8</td>
<td>.83±.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>52.8</td>
<td>-11±9</td>
<td>.64±.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>41.1</td>
<td>3.2±3.2</td>
<td>.92±.04</td>
</tr>
</tbody>
</table>

*Paw detector malfunction
In general it is not possible to reach any firm conclusions about changes in the peripheral blood flow from these data since there are obviously more variables involved than independent equations. Even if the tissues seen by the detectors homogeneously represented a two compartment leg, the problem is still not solved. In any circumstance that alters the shunt flow two factors can act, perhaps independently, to affect the end result. First, the distribution of arterial blood to the subject tissue may change. Secondly, the regional trapping fraction may change. In combination these two factors could even act in such a manner that a considerable regional change in MSP trapping could occur in the leg with no net change observed in the lung, e.g. a regional increase in MSP trapping could be balanced by a decrease in flow to that region. The above results are consistent however with the hypothesis that most AVA flow is associated with the skin and that epinephrine increases muscle blood flow relative to skin and/or closes down shunt vessels.

As mentioned above, total shunt flow after epinephrine is always lower than before. This is accompanied by variable effects locally in the leg. In three cases (exp. 1,2,14) thigh accumulation rate increased while paw accumulation rate decreased. Two cases (exp. 3,4) show thigh decrease with paw increase and two cases (exp. 5,7) show both paw and thigh increasing.

If we assume that fractional bypass of MSP in the leg is a measure of bypass blood flow, then nutritive flow is given by one minus the MSP bypass fraction. Table 4 shows
the ratio of nutritive flow fraction during epinephrine infusion to the pre-epinephrine value, based on the above assumption. These results are in reasonable
Table 4. Shunt flow fraction response to epinephrine infusion and corresponding change in nutritive flow fraction

<table>
<thead>
<tr>
<th>EXP</th>
<th>Shunt Fraction before epin. = ( F_1 )</th>
<th>Shunt Fraction during epin. = ( F_2 )</th>
<th>Nutritive Fraction Ratio = ( (1-F_2)/(1-F_1) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.679</td>
<td>*0.</td>
<td>3.1</td>
</tr>
<tr>
<td>2</td>
<td>0.328</td>
<td>0.061</td>
<td>1.4</td>
</tr>
<tr>
<td>3</td>
<td>0.934</td>
<td>0.305</td>
<td>10.5</td>
</tr>
<tr>
<td>4</td>
<td>0.535</td>
<td>0.040</td>
<td>2.1</td>
</tr>
<tr>
<td>5</td>
<td>0.906</td>
<td>0.244</td>
<td>8.0</td>
</tr>
<tr>
<td>7</td>
<td>0.637</td>
<td>0.207</td>
<td>2.2</td>
</tr>
<tr>
<td>8</td>
<td>0.830</td>
<td>0.196</td>
<td>4.7</td>
</tr>
<tr>
<td>12</td>
<td>0.528</td>
<td>*0.</td>
<td>2.1</td>
</tr>
<tr>
<td>14</td>
<td>0.411</td>
<td>0.032</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Avg. = 4.0

* Shunt fractions listed as negative in Table 3 are here taken as zero since the negativity is a statistical phenomenon.
qualitative agreement with the reports of Hippensteele (1967) and Goris (1972) who studied shunt flow in the dog's leg with diffusable ions. Hippensteele, using $^{24}$Na as the diffusible tracer, found that epinephrine infusion increased the volume of perfused tissue by factors of 2 to 3. Goris, using $^{133}$Xe, reports bypass blood flow fractions from 0.07 to 0.35. In two measurements on the effect of epinephrine, Goris found in one case an increase in nutritive flow and in the second case a decrease.

How reasonable is the assumption that MSP bypass corresponds to shunt or non-nutritional blood flow? Our observation of shunt flows as high as 90% at first seems surprising. We will show, first, that such results are not incompatible with other physiological data, but other arguments support the idea that shunt flow could be higher when measured with MSP as compared to diffusible ion measurements.

Spence et al. (1972) report average femoral artery blood flow in the dog to be 90 ml/min by direct flowmeter measurement. Friedman and Selkurt (1966) state that resting muscle blood flow is in the range 2-10 ml/min/100 gm. The observed weight of thigh muscle is about 500 gm in our dogs. Therefore 90% shunt flow would correspond to 81 ml/min shunt and 9 ml/min nutritive flow. For 500 gm muscle, the specific flow to muscle would be 1.8 ml/min/100 gm, just slightly below the reported range. Similarly 30% shunt implies 63 ml/min nutritive flow or about 13 ml/min/100 gm, again close to the accepted flow range. For this simple calculation, we assume that all muscle flow is nutritive and that nutritive skin flow is relatively negligible. Thus our observed shunt values are roughly in agreement with other data.
Two arguments may be advanced however which suggest that, if MSP and diffusible ions do not measure the same shunt flow, MSP will show a higher shunt flow fraction than will the diffusible ion. First, the work of Phibbs and Dong (1970) shows that MSP in blood vessels have some preferential axial accumulation and that this effect can become significant in small diameter vessels. We therefore suspect that capillaries which are fed primarily from the periphery of an arteriole would receive less than their expected number of MSP and the large diameter A-V shunts would receive correspondingly more. This effect would cause the MSP bypass fraction to be higher than the fractional bypass flow. Secondly, if the MSP passage is to indicate shunt flow, then all vessels that allow MSP passage must be impermeable to the diffusible ion. It is difficult to know whether this condition is met completely. If it is not, then shunt flow measured with MSP would again be higher.

VI.C. The effect of microsphere diameter

Arteriovenous shunts are postulated to be larger in diameter than capillaries. The shunts have a distribution of diameters and hence the measurement of shunt flow with microspheres is actually only a measurement of flow through vessels larger than the MSP. Lopez-Majano, Rhodes and Wagner (1969) report on leg shunting in the dog using 36 micron and 50 micron MSP, and macro-aggregated albumin (MAA). In all comparative studies the MAA showed the highest shunting, followed by the 36 micron MSP and then the 50 micron MSP. The differences were not extreme however, indicating that the distribution of AVA diameters is relatively broad and continuous - at least within this size range.
In order to investigate this with the infusion technique, three experiments were performed using a mixture of 15 and 35 micron MSP, each size with a different gamma-ray label. During each of these three experiments, an epinephrine infusion was made in order to see if any significant differences in shunt flow response to epinephrine could be attributed to sphere size. Results are summarized in Table 5.

From Table 5 we see that in general the 15 and 35 micron MSP behave quite similarly. Overall shunting is larger for the small spheres, as expected, but in two cases is barely significant (Experiments 4 and 5). The response of the two sizes during and after epinephrine is also similar with the possible exception of the paw ratios post-epinephrine to pre-epinephrine. Here we see that the ratio for 15 μm spheres is greater than that for 35 μm spheres. This would imply, in the paw, smaller diameter shunt vessels remain constricted for a longer time than large diameter vessels.

VI.D Relative shunt flow in muscle and skin

The next question to be discussed concerns the relative magnitude of shunt flow between skin and muscle tissue. The first investigation involved two experiments where blood flow to the paw was occluded with a pressure cuff placed distal to the ankle joint. In these cases, occlusion reduced the shunt flow fraction from 67% to 41% and from 47% to 24%. Although significant, these reductions were not as great as those typically observed with epinephrine. During occlusion no MSP accumulation was seen in the paw, while thigh accumulation increased by factors of 4.2 and 2.0. After release of the cuff, overall shunting returned to slightly below its
Table 5 - Comparative response of 15 and 35 MSP to epinephrine infusion

<table>
<thead>
<tr>
<th>EXP</th>
<th>Sphere size, microns</th>
<th>Lung</th>
<th>Paw Slope Ratios:</th>
<th>Thigh Slope Ratios:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre epin. shunt, %</td>
<td>During epin. shunt, %</td>
<td>Post epin.</td>
<td>During epin.</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>53.5</td>
<td>-4.0±3.8</td>
<td>.86±.05</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>49.6</td>
<td>4.8±2.4</td>
<td>.86±.04</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>90.6</td>
<td>24.4±2.2</td>
<td>.71±.02</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>85.1</td>
<td>21.1±1.9</td>
<td>.70±.02</td>
</tr>
<tr>
<td>6</td>
<td>15</td>
<td>38.3</td>
<td>15.9±2.9</td>
<td>1.00±.07</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>18.7</td>
<td>11.5±2.1</td>
<td>1.76±.07</td>
</tr>
</tbody>
</table>
original value, while paw accumulation was about double the pre-occlusion rate.

These results would support the idea that much of blood flow to the paw is shunt flow, but that significant shunt flow also occurs in proximal regions of the leg. The increase in paw accumulation of MSP post-occlusion is consistent with the concept of increased nutritive blood flow during reactive hyperemia.

Two other approaches were tried in order to assess the muscle vs. skin question. In one experiment, No. 15, surgical cut down exposed the femoral and saphenous arteries and their side branches. Arterial branches not accepted as supplying only muscle were ligated as shown in Fig. 9, and the infusion needle inserted at a point below the cranial femoral artery. A photograph of this procedure with arterial needle in place is shown in Fig. 10. As much as possible, the exposed area was kept covered with saline moistened gauze. The result of this infusion was that the overall shunt fraction was only 1.5 ± 0.1%. The infused leg was subsequently assayed for MSP content in three regions. These were: 1) the lower leg and paw, beneath the ankle joint, 2) the skin of the upper leg, and 3) the balance of the upper leg. Results of the assay showed the lower leg contained 2.7%, upper leg skin 1.7%, and balance of upper leg 95.5% of the total activity. Thus, we conclude that this method of MSP introduction enhances the proportion of flow to muscle. From the low shunt fraction, it appears that in this preparation at least, little if any shunting occurs in muscle.

In another experiment, No. 16, an arterial needle was inserted into each left and right femoral arteries in the usual percutaneous manner. In one leg an arterial MSP infusion was made during the
Fig. 9. Schematic representation of arterial infusion for preferential muscle supply.
Fig. 10. View of surgical cut-down region in experiment 15.
normal anesthetized state. This infusion was stopped, and then a venous epinephrine infusion was started, followed by MSP infusion of the other leg. A venous MSP infusion allowed calculation of overall shunt fraction for the two cases. Subsequent counting of tissue from the legs was performed to assay the relative distribution of MSP as was done in the previous experiment. The results are shown in Table 6. Experiments 15 and 16-B should both have increased blood flow in muscle relative to skin. The larger accumulation of MSP in the muscle tissue of the upper leg for both trials, relative to trial 16-A, indicates that this did occur. The low shunt values associated with high muscle flow suggest that the shunt flow primarily occurs in the skin and lower leg.
Table 6 - Shunt flow fractions and relative MSP concentrations from experiments 15 and 16

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Description</th>
<th>% Shunt</th>
<th>% MSP in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Skin of</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower leg upper leg less skin</td>
</tr>
<tr>
<td>15</td>
<td>Arterial cut-down and ligation</td>
<td>1.5±0.1</td>
<td>2.7 1.7 95.4</td>
</tr>
<tr>
<td>16-A</td>
<td>Normal state</td>
<td>76±4</td>
<td>17.5 5.9 76.5</td>
</tr>
<tr>
<td>16-B</td>
<td>With epinephrine</td>
<td>12±1.6</td>
<td>1.9 0.9 97.2</td>
</tr>
</tbody>
</table>
Summary

Measurement of shunt blood flow fraction in the dog's leg has been accomplished by arterial infusion of MSP. Using external radiation detectors to monitor the number of MSP bypassing the leg and reaching the lung, we find that the bypass fraction of MSP is constant in time at least for periods of 8-12 minutes. Additionally the local accumulation rate of MSP in gross areas of the leg such as thigh and paw is also constant. The observed bypass fractions for 15 μ MSP in the normal anesthetized dog ranged from 33% to 93%.

The infusion technique is valuable in following the response of the shunt flow to physiological manipulations since both global and local variations are measured continuously. We have observed the changes in shunt flow caused by epinephrine infusion. The effects are quite striking. Overall leg shunting decreases greatly, sometimes to zero. Local nutritive flow as determined by leg accumulation of MSP also is changed; in the paw nutritive flow decreases while in the muscular thigh region nutritive flow increases.

It is generally accepted that epinephrine reduces skin blood flow and increases muscle flow. The presence of large diameter A-V anastomoses in skin is also well known; our work indicates that few if any such anatomical shunts exist in muscle tissue. Our results with epinephrine are consistent with the above facts and thus support the hypothesis that MSP shunting corresponds to non-nutritive shunt blood flow.

The anatomical location of the shunts was studied in several ways. When all paw circulation was occluded with a pressure cuff, overall shunt flow decreased, but not as drastically as with
epinephrine infusion. Since epinephrine acts on the entire leg while the cuff affects only the paw, this result implies that significant shunting occurs somewhere in the upper leg, as well as in the paw.

The fact that this upper leg shunting is primarily associated with skin was shown in an experiment where all arteries not accepted as supplying only muscle were ligated before MSP infusion. The shunt fraction in this case was only 1.5% compared to the usual shunt range of about 30 to 90%. This observation shows that most if not all shunting of MSP occurs in skin and paw and very little in muscle.
Acknowledgments

I wish to acknowledge support of this work from the USAEC Health Physics Fellowship, administered by Oak Ridge Associated Universities and from USPHS Training Grant No. 2 T01 GM0829 from the National Institute of General Medical Sciences. The use of the laboratory and support facilities of the Lawrence Berkeley Laboratory, operated by the University of California for the USAEC, is also gratefully acknowledged.

Among the many faculty who have influenced my studies on the Berkeley campus, I would especially like to acknowledge my dissertation committee members, Dr. Ernest Dobson, Dr. Julien Hoffman and Dr. Hardin Jones. Their interest, patience and humanity were a continuing aid during this investigation.
Appendix I - Weighted Linear Least Squares Fitting

I have made extensive use of linear least squares fitting in the analysis of my experimental data. Therefore I wish to discuss formally the statistical theory involved, with careful attention to the assumptions and limitations of the theory.

The following notations and definitions will be used

\[ Y_i = \text{one of a set of experimental values, which is subject to statistical variation} \]
\[ X_i = \text{the independent variable associated with } Y_i, \text{not subject to variation} \]
\[ \beta_j = \text{one of the functional parameters relating } Y_i \text{ to } X_i \]
\[ \epsilon_i = \text{the statistical "error" associated with } Y_i \]
\[ \hat{\beta}_j = \text{the statistical estimator of } \beta_j \]

Then with the hypothesis that the relation between \( Y \) and \( X \) is linear, we may write

\[ Y_i = \beta_1 + \beta_2 X_i + \epsilon_i \quad (1) \]

It is assumed that the \( \epsilon_i \) are uncorrelated and have a normal distribution with mean zero and common variance \( \sigma^2 \). The set of \( n \) linear equations similar to (1) may be expressed in matrix form:

\[ Y = X\hat{\beta} + \epsilon \quad (2) \]

and pre-multiplying by \( X^T \), the transpose of \( X^T \)

\[ X^TY = X\beta + \epsilon \quad (3) \]

We now wish to find the vector \( \hat{\beta} \) such that the sum of squares of \( \epsilon_i \) is a minimum. It can be shown that this is equivalent to requiring \( X\epsilon \) be equal to zero. Thus

\[ \hat{\beta} = (XX^T)^{-1} XY \quad (4) \]

Now \( \hat{\beta} \) is a random vector estimating \( \beta \). An expression for the variance of the \( \hat{\beta}_1 \) can be derived with the help of defining
the covariance matrix, $S_{ij}$, of a random vector $\mathbf{w}$ where the elements of $S_{ij}$ are

$$S_{ij} = \text{Cov}(w_i, w_j)$$

and note that $s_{ii} = \text{Var}(w_i)$. Then it can be shown that if $V = A\mathbf{w}$ where $A$ is a matrix of constants then

$$S_V = A S_W A'$$

where $A'$ is the transpose of $A$.

Then from eq. 4, noting that $(XX')^{-1} X$ is a constant matrix:

$$S_{\beta} = (XX')^{-1} X S_Y (XX')^{-1} X$$

$$= (XX')^{-1} X S_Y X' (XX')^{-1}$$

Since however $S_Y = S_\varepsilon \equiv \sigma^2 I$, we have

$$S_{\beta} = \sigma^2 (XX')^{-1}$$

which expresses the variances of the $\beta_i$.

In our experimental data, the count rates $(Y_i)$ do not have a common variance $\sigma^2$ as was required in the above development. Since $N$, the number of counts recorded, has a Poisson distribution,

$$\text{Var}(N) = N$$

The case of unequal variances may be handled by a simple transformation of variables. We have that

$$S_Y = B \neq \sigma^2 I$$

where $B$ is known from eq. 9, and we desire a linear transformation $\mathbf{z} = A\mathbf{y}$ such that

$$S_z = A B A' = I$$

With this transformation, it follows that the expression for $\beta$, eq. 4, becomes

$$\hat{\beta} = \left[(XX'AX')^{-1} \right] X A' AY$$

(12)
From eq. 11, we have
\[ B = A^{-1} (A')^{-1} = (A'A)^{-1} \] (13)
and substituting in eq. (12)
\[ \hat{\beta} = \left[ X B^{-1} X' \right]^{-1} X B^{-1} Y \] (14)
This is the form of weighting used in our data analysis.

The specific form of \( B \), the covariance matrix of the experimental count rate values is as follows. First, \( B \) is a diagonal matrix since the set of count rate values are assumed statistically independent. The diagonal terms are the variances of the corresponding count rates. Let

\[ N = \text{observed number of counts, a statistical random variable with Poisson distribution} \]
\[ T = \text{the time interval during which counts are accumulated, not subject to statistical variation} \]
\[ R = \frac{N}{T} = \text{the count rate} \]

Then \[ \text{Var } R = \text{Var } \left( \frac{N}{T} \right) \]
\[ = \frac{1}{T^2} \text{Var } N = \frac{N}{T^2} \]
\[ = \frac{R}{T} \]

Thus the element of \( B \) corresponding to count rate \( R_i \) determined during time interval \( T_i \) is
\[ b_{ii} = \frac{R_i}{T_i} \]

The question remains as to the validity of the hypothesis that the relation between \( Y \) and \( X \) is indeed linear. We have used a "goodness of fit" parameter for this determination, as well as visual inspection of the residuals for obvious non-linearity. In the linear case, the residual for any one data point is given by.
\[ r_i = \hat{\beta}_1 + \hat{\beta}_2 x_i - y_i \]  

(15)

For the weighted least squares analysis (eq. (14)), the weighted sum of squares of the \( n \) residuals has an expected value of \( n-2 \). Thus we have used a goodness of fit factor defined as

\[
G.O.F. = \frac{\sum_{i=1}^{p} \frac{r_i^2}{y_i}}{n-2}
\]

(16)

Values of G.O.F. near unity imply that the linear hypothesis is true. Values above about 1.5 have been accompanied by patterns in the plotted residuals, indicating non-linear relations.

The use of weighted least squares fitting is preferred when the variances of the data points have a wide range. For example, a deviation of 50 counts from the fitted line would be reasonable for a data point of 2500 counts. The same 50 count deviation would be unacceptable for a data point of 100 counts. Standard least squares fitting accepts either of the above cases as equally valid, while obviously the deviation (in counts) should be smaller for the 100 count point than for the 2500 count point. Weighted least squares fitting accomplishes this and hence provides a better statistical estimate of the fitted curve parameters.
Appendix II - Computer program listings

The following programs were used for data analysis as detailed in part D of the materials and methods section of this report. We wrote the programs in FOCAL-12, a modified version of the FOCAL language. The advantages of FOCAL-12 include: 1) data stored on magnetic tape are accessible directly, 2) FOCAL-12 programs are also stored on tape, allowing use of sub-routines or "chaining" of related programs and 3) graphic output can be generated and photographed for reference.

Program START:

```
01·10 E
01·20 L 0, F1, F, #700, 0
01·30 L 0, F2, U, #0, 1
01·31 L 0, F3, F, #677, 0
01·32 S F3(17)=1
01·35 A "RUN NO. ", RN; S F3(19)=RN; RN=16*(RN-1)*256; T !
01·40 A "# EPOCHS", NE; "# CHANS", NC
01·42 S F3(0)=NE; S F3(1)=NC
01·50 S X=NE*(NC+1)-1
01·60 F I=0; X; S F1(I)=F2(I+6+RN)
01·64 S NE=NE-1; S NC=NC+1
01·65 F I=0; NE; S F1(NC+1)=F1(NC+1)/3000
01·70 L C, F1; L C, F2; L C, F3
01·75 T ! ! !
01·80 Q
```
Program DPLOT:

```
01.05 E
01.10 L O,F1,F,#700,0
01.11 L O,F3,F,#677,0
01.20 A "CHANNEL",CH
01.22 S NE=F3(0);S NC=F3(1)
01.30 S NE=NE-1;S NC=NC+1
01.35 S T=0;S B=0;S TT=0
01.40 F I=0;NE;D 2
01.45 D 5
01.50 F I=0;NE;D 3
01.51 A !,"CHANGE SCALES ?",CS;I (CS-20) 1.55;
01.52 O C
01.53 A !,"X & Y FACTORS",XF,YF
01.54 S TT=TT/XF;S E=B/YF;D 5;S T=0;G 1.50
01.55 A !!!,"MORE PLOTS ?",M;I (20-M) 4.1;
01.60 L C,F1;L C,F3
01.65 O C
01.70 T !!!;Q
02.10 S TT=TT+F1(I*NC)
02.20 S RT=F1(I*NC+CH)/F1(I*NC)
02.30 I (K-T-B) 2.4;S B=RT
02.40 C
03.10 S X=I*NC
03.20 S T=T+F1(X)
03.30 S RT=F1(X+CH)/F1(X)
03.40 S H=FDIS(1.38*T/TT,RT/B);O D
04.10 O C
04.20 A !,"CHANNEL",CH
04.30 G 1.35
05.10 O S;T % 5.0," "" "",B," CFM"
05.12 T I,%2.0,"CHANNEL "",CH," HUN ",F3(19)
05.15 F I=0,27;T !
05.17 F I=0,11;T " 
05.20 T % 5.2;TT," MIN ";O T
05.25 F I=0,2;FITR(TT);F J=0,3;S H=FDIS(1.38*I/TT,J/100)
05.30 F I=0,10;FITR(TT);F J=4,7;S H=FDIS(1.38*I/TT,J/100)
```
Program LINE:

01.10 E
01.15 0 C
01.20 L 0xF1,F,#700,0
01.22 L 0xF2,F,#677,0
01.24 L 0xF3,F,#700,1
01.26 I (F8(17)) 1.27,1.30,1.30
01.27 S CH=F8(16); S TS=F8(15); S TF=F8(16); S F2(17)=1; G 1.35
01.30 A !*"CHANNEL",CH!*"START TIME",TS!*"END TIME",TF!*
01.35 F K=0;4; S SM(K)=0
01.40 S T=0; S NC=F8(1)+1; S I=-1; S J=-1; G 1.45
01.43 S T=T+5*T/T
01.45 S I=I+1; S TT=F1(I*NC); S T=T+5*T/T
01.50 I (T-TS) 1.43; J
01.55 S J=J+1; S F3(4*J)=T
01.56 S F3(4*J+1)=F1(I*NC+CH/T/T
01.58 S R=F3(4*J+1); S F3(4*J+2)=T
01.60 S SM(0)=SM(0)*T/T
01.64 S SM(1)=SM(1)+T/T
01.68 S SM(2)=SM(2)+T/T/R
01.72 S SM(3)=SM(3)+T/T*T/R
01.76 S SM(4)=SM(4)+T/T*T/R
01.80 S F3(4*J+3)=F3(T/R/T/T
01.82 S T=T+5*T/T
01.85 S I=I+1; S TT=F1(I*NC
01.90 S T=T+5*T/T
01.95 I (T-TF) 1.55;

02.10 S DT=1/<SM(3)*SM(2)-SM(4)*SM(4)>
02.20 S SL=DT<SM(2)*SM(1)-SM(0)*SM(4)>
02.25 T !%5.1=","SLOPE IS ","SL," CFM/MIN"!"
02.26 T " +=","FSQT(DT*SM(2) "," CFM/MIN"!"
02.30 S IN=DT<SM(0)*SM(3)-SM(1)*SM(4)>
02.32 T %5.1="INTER. IS ","IN"!
02.35 F K=0;4; S F2(6+K)=SM(K
02.36 S F2(11)=I; S F2(12)=J
02.38 S F2(13)=SL; S F2(14)=IN
02.40 S F2(15)=TS; S F2(16)=TF
02.45 S F2(18)=Ch
02.50 L C,F1:L C,F2:L C,F3
02.60 L G,SLINE1,0
Program LINE 1:

01.10 E
01.20 L 0,F1,F,700,0
01.22 L 0,F2,F,777,0
01.24 L 0,F3,F,700,1
01.30 S I=F2(11);S J=F2(12)
01.32 S SL=F2(13);S IN=F2(14)
01.34 S TS=F2(15);S TF=F2(16)
01.36 S CH=F2(18)
01.40 S SS=0;S AX=0;F K=0;J;D 3
01.50 D 4
01.55 S X=I-1-J+JJ;S XX=F3(4*JJ+2)*<IN+SL>F3(4*JJ>)
01.60 S F1<9*X+CH>=XX
01.62 O C
01.63 L C,F1;L C,F2;L C,F3
01.65 L G,SLINE=0

03.10 S RS=F3(4*K+1)-IN-SL*F3(4*K)
03.15 S RS=RS/F3(4*K+3);S F3(4*K+3)=RS
03.17 S RS=ABS(RS)
03.18 S SS=SS+RS*RS
03.20 I <AX-RS> 3.25,3.30,3.30
03.25 S AX=RS;S JJ=K
03.30 C

04.10 T !"RES FOR POINT =",%3*0, JJ," IS",%4*2, AX
04.11 T " SIGMA UNITS"
04.12 T !"GOOD OF FIT =",%SS/J-1"," FOR ",J+1," POINTS"
04.20 F K=0,1371S H=FDIS(K/100,.5)
04.30 S M=TF-TS
04.35 O SIT %2*0,"CHANNEL ",F2(18)," RUN ",F2(19),!
04.36 T %3*1,TS=" TO ",TF," MINUTES",!,"+- 5 SIGMA F"," S=
04.37 O T
04.40 F K=0, J; S H=FDIS<1.38>(F3(4*K)-TS)/M,5+F3(4*K+3)/10>
04.45 A 1,"DROP WORST POI N T ? ",DR
04.47 I (DR-20) 4.50,4.66,4.66
04.50 A 1,"CHANGE SCALES ? ",DR
04.52 I (DR-20) 4.6,4.55,4.55
04.55 A 1,"SCALE FACTOR ? ",SF;S AX=AX*SF;O C;G 4.2
04.60 L C,F1;L C,F2;L C,F3
04.62 Q
04.66 S F2(17)=-1
Program XTAI-K:

01.05 E
01.10 L 0,F3,F,677,0
01.15 L 0,F2,F,700,0
01.20 S Z=F3(0); S NC=F3(1)+1
01.30 A "CH. TO BE FIXED",C1
01.35 A "XTALK FACTOR",XF
01.40 A "CH. FIXING",C2
01.45 A "CPM BKGD THIS CH",B2
01.55 F I=0,Z-1; D 2
01.70 L C,F2;L C,F3
01.72 Q

02.10 S DU=F2(NC+I+C2)-B2+F2(NC+I)
02.20 S F2(NC+I+C1)=F2(NC+I+C1)-XF*DU
Appendix III - System dead time measurements

In any radiation detector counting system, it is necessary to consider the effects of "dead time" on the observed count rates. A brief discussion of this problem is given by Evans (1955). Dead time results from the failure of the detection system to record some actual events because it is busy recording an earlier event and hence is unable to respond.

A common method of measuring dead time involves the use of two radiation sources (Evans, 1955). The sources are counted separately and then together. The sum of the individual counts is greater than the simultaneous count because of dead time count losses, and this difference allows an approximate calculation of dead time.

An alternative and possibly more useful measurement can be made by following the decay of a short-lived isotope. As this source decayed, the count rate would eventually become low enough that dead time losses were negligible and the rate would then decrease as a simple exponential. Back extrapolation of this decay curve would give a graphical presentation of observed and true count rates for the range where dead time losses were significant.

This measurement was made using $^{68}\text{Ga}$ ($T_{1/2} = 68.3\text{ min}$). Six detectors were connected in parallel to the computer interface and sources placed so that each detector started with a count rate of about 15,000 sec$^{-1}$. Count rates were then measured periodically for six hours. The results are shown in Fig. 11 where the total count rate for all six detectors is plotted against time.
Fig. 11. Observed count rates and fitted curve for $^{68}\text{Ga}$ decay.

$^{68}\text{Ga}$ decay curve

$T_{1/2} = 68.3\text{ min}$

Region of fitting to corrected points (see text)
It was felt that the system dead time was probably about 3 μsec. This was because the storage of an event requires two computer memory cycles and a memory cycle is nominally 1.5 μsec. Therefore we made a correction to the observed count rates, assuming 3 μsec dead time, and then compared the resulting half-life with the accepted value. For small corrections, e.g. less than 5%, Evans shows the following relation

\[ N = n(1 + np) \]

where \( N \) = true counting rate

\( n \) = observed counting rate

\( p \) = dead time

Our corrections were made on all points where the count rate was less than 13,000 sec\(^{-1}\) corresponding to a maximum 4% adjustment.

An unweighted least squares fit to the 14 corrected data points was made, assuming simple exponential decay. The calculated half life was 68.3 min., and the RMS deviation of the data from the fitted line was 0.4%.

This result was in exact agreement with the best accepted value for \(^{68}\)Ga (Lederer et al. (1967)). We, therefore, conclude that the system dead time is indeed 3 μsec. The maximum correction in our M6P experiments for this dead time was typically about 0.5%, which was judged insignificant, and hence no dead time corrections were made.
References


Grant, R.T. and E.F. Bland, Observations on arterio-venous anastomoses in human skin and in the bird's foot with special reference to the reaction to cold, Heart 15:385 (1931)


Kety, S.S., Measurement of Regional Circulation by the Local Clearance of Radioactive Sodium, Am Heart J. 38:321 (1949)


Mendelowitz, M., Cardiovascular Shunts, Amer. J. Med. 22 (1957).


LEGAL NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Atomic Energy Commission, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.