PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **181**, 338–344 (1986)

Tibial Nerve and Deep Fibular Nerve Effects on Venous and Extravascular Volumes (42262)

CARLETON H. BAKER, DARRELL L. DAVIS, AND E. T. SUTTON

Department of Physiology, College of Medicine, University of South Florida, Tampa, Florida 33612

Abstract. The innervation of the vasculature of the dog hindpaw separately controls the series and parallel coupled vessels by means of the tibial, deep fibular, and superficial fibular nerves. The latter primarily affects veins. The venous effects of the tibial and deep fibular nerves have not been adequately defined. The right hindpaw of anesthetized dogs was vascularly and neurally isolated in a volume recorder. The animals were heparinized and the preparation autoperfused (constant pressure). Total tissue volume changes were determined by the volume recorder. Total vascular volume changes were calculated from changes in paw ^{51}Cr-red blood cell radioactivity measured by a scintillation detector. Arterial pressure and paw blood flow were monitored. The tibial and deep fibular nerves were each separately stimulated at 1, 5, and 15 Hz. Deep fibular nerve stimulations resulted in progressively significant increases in precapillary flow resistance. Vascular and tissue volumes decreased with stimulation frequency but vascular volume decreased significantly less than tissue volume change. Tibial nerve stimulation resulted in significant precapillary resistance increases. Vascular and tissue volumes decreased by similar amounts. Thus, deep fibular nerve stimulation causes passive decrease in venous volume, reduced capillary pressure, and fluid absorption. Tibial nerve stimulation causes active arterial and venous constriction maintaining capillary pressure with minimal fluid transfer. © 1986 Society for Experimental Biology and Medicine.

Zimmerman (1, 2) as well as Davis and Hammond (3), Hammond *et al.* (4), and Davis (5) have demonstrated that the vasculature of the dog hindpaw is innervated by three nerves with differing influences on the series coupled vessels. It has also been demonstrated that to a large degree these nerves differentially innervate the parallel coupled capillary or nutritional circuit and the arteriovenous anastomoses (AVA) or nonnutritional circuits (6–9). The superficial fibular nerve primarily influences the venous segments with some effects on the arterioles and AVA. The deep fibular nerve innervates the arteries and AVA and to a minimal degree the arteriolar segments. The tibial nerve innervates the AVA, and minimally innervates the arteries and arterioles. It has been extrapolated from pressure-flow data, capillary function data, and bolus injection indicator dilution determinations of active or circulating vascular volume that the deep fibular nerve has little effect on veins and that the tibial nerve seemed to cause some direct constriction of veins. Since active volume is usually smaller than total vascular volume (12, 13), the present study was designed to establish whether changes in venous volume during stimulation of the deep fibular nerve or the tibial nerve were due to passive or active changes in venous capacity.

Total tissue volume changes of the dog hindpaw were determined by means of plethysmography and total vascular volume changes were calculated from changes in paw ^{51}Cr-red blood cell (RBC) radioactivity measured by a scintillation detector. Arterial perfusion pressure and paw blood flow were also monitored and blood flow resistance was calculated.

Materials and Methods. Twenty mongrel dogs averaging 19 kg in weight were pretreated with 10 mg/kg morphine sulfate and anesthetized with 15–20 mg/kg sodium pentobarbital. The right hindpaw (ave wt = 280 g) was neurally and vascularly isolated at the ankle joint (Fig. 1). The cranial tibial artery, saphenous vein, and the tibial, superficial, and deep fibular nerves were isolated. The nerves were doubly ligated and sectioned so that the effects of stimulation of the peripheral stumps of the tibial and deep fibular nerves would not be modified by the other nerves. The skin flap produced by the isolation procedures was used as a seal for an air-filled volume recorder (plethysmograph).

The animals were administered 10 mg/kg heparin intravenously. The outflow from the saphenous vein was returned to the femoral vein, thus returning all blood flow from the paw directly to the animal. The paw was per-

0037-9727/86 $1.50
Copyright © 1986 by the Society for Experimental Biology and Medicine.
All rights reserved.

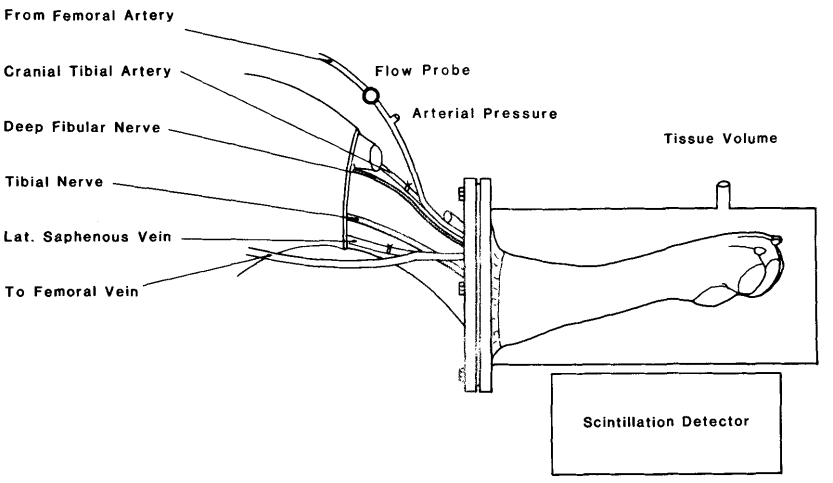

FIG. 1. Schematic diagram of the experimental arrangement for the dog hindpaw preparation.

fused through the cranial tibial artery from the femoral artery. The flow was determined by the dog's arterial pressure and the paw resistance. Arterial pressure and flow were measured from the cannula in the cranial tibial artery. During the cannulation procedure, blood flow to the hindpaw was interrupted for less than 1 min.

Stimulations of the deep fibular nerve (40 V, 0.3-msec duration) and the tibial nerve (60 V, 0.3-msec duration) were made at 1–3, 5–8, and 14–15 Hz for a period of 1 min. The stimulation voltage was different for the two nerves as the tibial nerve had a higher threshold and maximal value; therefore, the supra maximal stimulation strength was greater than for the deep fibular nerve. Also, the stimulation frequencies are reported as a range since the first two preparations were stimulated at 3, 8, and 15 Hz. All of the other preparations were stimulated at 1, 5, and 14 Hz. It was observed that there were no real differences in the responses within the ranges. The stimulations of the two nerves were done in a random manner. The frequencies used in each experiment were determined so that a minimal, moderate, and a maximal response could be obtained. Elapsed time of each stimulation of each nerve was 1 min. Only experiments in which the blood flow decreased to a new level which was maintained for the remainder of the stimulation period were used. At least 10 min was allowed between each stimulation to allow each measured parameter to return to the control level. There were no significant differences between the controls for the various stimulations and they were therefore combined.

The duration of the experiments was variable. Sufficient postsurgical time was allowed for the animals to stabilize and the hindpaws to become isovolumetric. It was unnecessary to immobilize the animals with a curare-like drug since stimulation of the nerves rarely elicited movement of the paw. In those cases where movement did occur the data were not different from paws that did not show contractions.

All measurements were made during control periods and during the entire time of each period of nerve stimulation, and were recorded on a DR-8 Electronics for Medicine recorder. Arterial pressure was monitored using Statham P23D transducers. Total paw volume changes (tissue volume) were measured with the air-filled plethysmograph connected to a Statham P23-3D-300 pressure transducer. The sensitivity of the system was 4 cm of recorder deflection per milliliter change in paw volume. Arterial inflow was monitored with a Carolina Medical electromagnetic flowmeter with a cannulating probe placed in the arterial circuit.

Vascular volume changes of the paw were measured using the γ emission of red cells-^{51}Cr. The red cells were labeled as previously reported (10), injected intravenously, and allowed to mix in the entire blood volume at least 30 min. We have shown that after this

period of time the counts per minute per milliliter of blood were constant. As shown in Fig. 1 a columnated γ scintillation detector was directed at the paw contained within the plethysmograph. The detector was connected to a ratemeter which in turn was connected to the recorder. Sufficient indicator was injected to yield approximately 5000 cpm from red cells-^{51}Cr in the paw. Since the radioactivity is limited to the blood or vascular space, changes in the level of radioactivity in the paw would represent changes in the volume of blood contained within the paw. The vascular volume changes resulting from stimulations of the nerves were calculated from the changes in paw radioactivity determined by the detector and ratemeter and determination of the total blood volume in the paw. The total blood volume in the paw was determined at the end of the experiment by ligation of the tibial artery and the saphenous vein; the paw was then removed from the dog and digested in concentrated potassium hydroxide contained in a 2-liter closed container. Also the counts per minute per milliliter of venous blood were measured in a well detector. A sample of venous blood was taken from the paw venous outflow tubing just before each stimulation. The calculations necessary for determining the changes in vascular volume in the paw are as follows:

$$\frac{[(\text{beaker} + \text{KOH} + \text{dissolved paw})\text{wt} - (\text{beaker})\text{wt}](\text{g})}{\text{specific gravity}} = \text{total volume (ml)}$$

$$\text{total volume} \times (\text{cpm/ml} - \text{background cpm}) = \text{total cpm from dog paw}$$

$$\text{total cpm} \div \text{decay factor of isotope} = \text{total cpm day of experiment}$$

$$\text{total cpm} \div (\text{cpm/ml blood} - \text{background}) = \text{ml of blood in dog paw}$$

$$\text{blood volume} \times \frac{\text{cpm during stimulation}}{\text{cpm at control}} = \text{blood volume change (ml)}$$

Blood flow resistance (mm Hg/ml $\cdot$ min^{-1}) was calculated as the arterial pressure (mm Hg) divided by the paw blood flow (ml/min). The changes in vascular volume were compared to each other as well as to changes in tissue volume as measured with the volume recorder. All data are reported with standard error of the means (SEM). Appropriately related values were evaluated with a paired *t* test.

Results. *Deep fibular nerve stimulations.* The hindpaws were perfused at the dog's arterial pressure which did not significantly change throughout the duration of the experiments (Fig. 3) and during the periods of nerve stimulation (Fig. 2). Figure 2 shows original records of changes in blood flow, blood volume, and tissue volume in one hindpaw when the deep fibular nerve was stimulated at 1, 5, and 14 Hz. The changes at 1 Hz were small, but pronounced decreases occurred in all parameters at 5 Hz. Large changes occurred at 14 Hz. Figure 3 shows the average values for the eight experiments with deep fibular nerve stimulation. The arterial pressure did not significantly change during the period of the experiments. However, blood flow, 34.6 $\pm$ 1.1 ml/min at control, decreased with increasing stimulation. The peak resistance at 14–15 Hz was 36.6 $\pm$ 2.2 as compared to the control value of 3.4 $\pm$ 0.1 mm Hg/ml $\cdot$ min^{-1}. The flows at 5 to 8 and 14 to 15 Hz averaged 20.7 $\pm$ 3.4 and 3.3 $\pm$ 1.8 ml/min, respectively, and were significantly lower than during control ($P < 0.01$). The control blood volume of the paw averaged 9.8 $\pm$ 0.7 ml. The volume of the paw contained within the plethysmograph averaged 247 $\pm$ 21 ml. The tissue volume and the blood volume decreased with increasing stimulation frequency. However, the tissue volume decreased (1.04 $\pm$ 0.23 and 2.53 $\pm$ 0.17 ml) significantly more ($P < 0.05$) than the blood volume (0.52 $\pm$ 0.23 and 1.66 $\pm$ 0.13 ml) at 5–8 and 14–15 Hz, respectively. A large dose of norepinephrine (1 μg ia) decreased tissue volume 1.87 $\pm$ 0.22 ml and blood volume 1.17 $\pm$ 0.15 ml. Therefore stimulation of the deep fibular nerve reduced tissue volume and blood volume more than norepinephrine.

Tibial nerve stimulation. Figure 4 shows

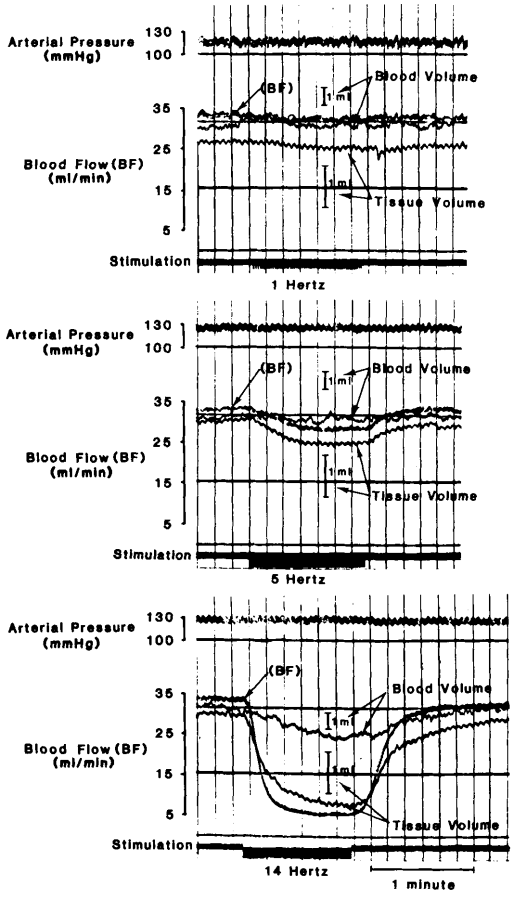

FIG. 2. Records of the responses to stimulation of the deep fibular nerve of one representative hindpaw at 1, 5, and 14 Hz. On each record the tracings from the top down are arterial pressure, blood flow, ^{51}Cr-RBC blood volume, tissue volume by plethysmography, and nerve stimulation. The vertical lines are 10 sec apart. One milliliter calibrations are for blood volume (top) and tissue volume (below).

original records of changes in blood flow, blood volume, and tissue volume with tibial nerve stimulation at 1, 5, and 14 Hz in the same hindpaw as used in Fig. 2. Flow, blood, and tissue volumes each decreased progressively with increasing stimulus frequency. The decreases in blood flow were rapid, reaching a plateau in 10 to 20 sec, while the blood and tissue volume responses were slower, requiring 30 to 50 sec to reach the maximum response at each stimulation frequency.

Figure 5 presents the average responses to tibial nerve stimulation. The arterial pressure did not change significantly from control as a result of the three stimulation frequencies. However, blood flow decreased significantly ($P < 0.05$) from control (34.6 ± 1.4 ml/min) at each stimulation frequency (30.1 ± 1.9, 25.5 ± 1.9, 15.5 ± 1.6 ml/min) and calculated resistance increased. However, the peak resistance was less ($P < 0.05$) than found with deep fibular nerve stimulation at the highest frequency (7.5 ± 1.6 vs 36.6 ± 2.2 mm Hg/ml · min^{-1}). The tissue volume and blood volume of the paws each decreased with increasing stimulation frequency. It is noteworthy that both values decreased by the same amounts and were not significantly different at the highest stimulation frequency; 1.76 ± 0.22 ml of tissue volume vs 2.22 ± 0.32 ml of blood volume. Therefore, tibial nerve stimulation reduced tissue volume by an amount comparable to the maximal decrease by norepinephrine stimulation.

Discussion. The control of the capacitance vessels of the dog hindpaw has been incompletely defined. It was reported some years ago

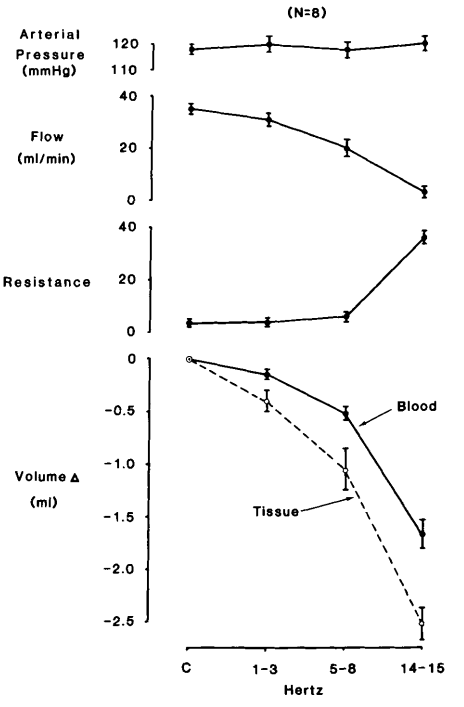

FIG. 3. Mean values for arterial pressure, blood flow, resistance, and tissue and blood volume changes at control and during stimulation of the deep fibular nerve. Brackets indicate SEM.

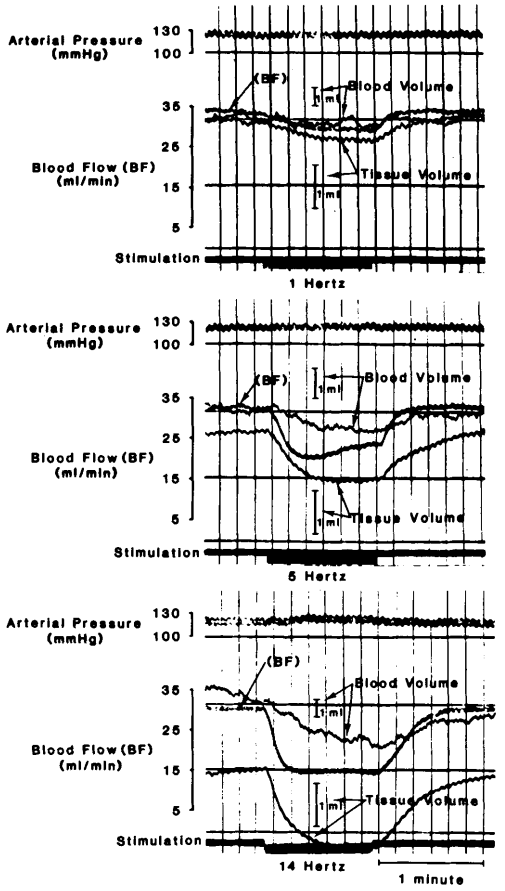

FIG. 4. Records of the responses to stimulation of the tibial nerve of one representative hindpaw at 1, 5, and 14 Hz. The tracings are in the same order as in Fig. 2 with the exception that the ^{51}Cr-RBC blood volume trace at 14 Hz was moved above the blood flow record for better separation and to avoid disturbing the calibration of the blood flow tracing.

that the series and parallel coupled vessels in this tissue were generally separately innervated by three nerves (1–5). However, it has become evident with further experimentation that there was some overlapping of influences by the three nerves (7–9). The control of the veins was considered to be primarily by means of the superficial fibular nerve. However, it was not clear if the reductions in venous capacity with deep fibular nerve stimulation and tibial nerve stimulation were due to passive flow effects or active constriction of the veins. In previous reports we have shown that active or circulating vascular volume as determined by the bolus injection indicator dilution technique (11) decreased (6–9) but as we have also shown previously (12, 13) active and total vascular volume do not usually change directionally and quantitatively the same. Therefore, changes in total vascular volume as determined by changes in radioactivity of ^{51}Cr-RBC along with changes in total tissue volume (plethysmography) should give us necessary information on this point. We have used this method previously to assess changes in total vascular volume of tissues (14, 15).

Stimulation of the deep fibular nerve increased vascular resistance gradually at 1 and 5 Hz but at 14 Hz resistance increased by about seven times the level at 5 Hz. Also the deep fibular nerve stimulations resulted in greater decreases in tissue volume than vascular volume at all stimulation frequencies. This can be explained by increased precapillary resistance as noted above, and apparently none or possibly a proportionately smaller increase in venous resistance. This would result

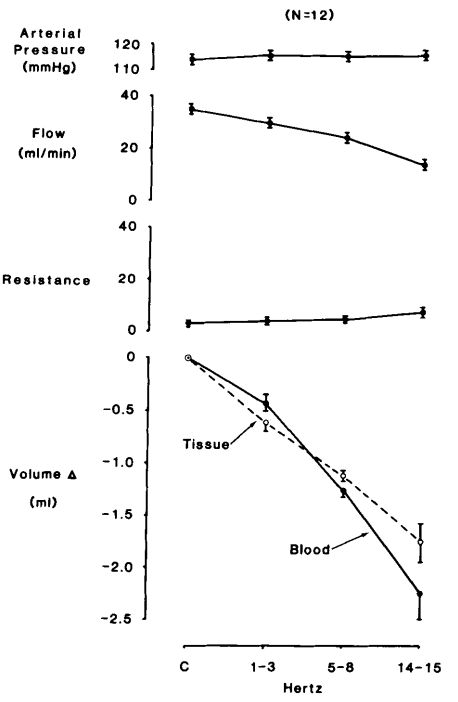

FIG. 5. Mean values for arterial pressure, blood flow, resistance, and tissue and blood volume changes at control and during stimulation of the tibial nerve. Brackets indicate SEM.

in a decrease in capillary hydrostatic pressure allowing absorption of extravascular fluid across the capillary, reducing tissue volume due to two components: (i) reduced extravascular fluid volume and (ii) passive reduction in venous volume consequent to the reduced blood inflow because of the markedly elevated precapillary resistance. The difference between the decrease in tissue volume and vascular volume would be the volume of absorbed extravascular fluid.

With tibial nerve stimulation the resistance to blood flow increased by a significantly smaller amount than with deep fibular nerve stimulation, especially at 14 Hz. Stimulations of the tibial nerve resulted in equal decreases in tissue and vascular volumes. Since the precapillary resistance increases were moderate and flow was, therefore, maintained at a higher level than during deep fibular nerve stimulation, capillary hydrostatic pressure would not decrease as much with tibial nerve stimulation due to this mechanism. Also since vascular volume and tissue volume decreased by the same amount at all stimulation frequencies, venous constriction could have occurred so that capillary hydrostatic pressure would be maintained and so there would be no net absorption or filtration of fluid across the capillary membrane. Therefore, the decreases both in total vascular volume as well as tissue volume consequent to tibial nerve stimulation would be due to the decrease in vascular volume, which would by necessity be the result of a proportionate active contraction of the smooth muscle of the veins reducing their capacity and increasing the postcapillary resistance.

Zimmerman (1) did not find an increase in venous resistance with tibial stimulation. There are several possible reasons for this discrepancy between his results and those in our experiments. One difference is that Zimmerman (1) used constant flow perfusion, whereas constant pressure perfusion was used in our studies. With constant flow the changes in volume and resistance are much smaller than with constant pressure (8). This is presumably due to the greater volume of blood being forced into the venous system with constant flow, thus not allowing the veins to reduce their capacity. Another possible explanation is that the reduction of vascular and tissue volume caused by venous constriction in our studies could have been downstream from the segment where Zimmerman (1) measured small vein pressure.

The number of tibial nerve stimulations differed from the number of deep fibular nerve stimulations due to technical difficulties with some of the preparations. Therefore, it is difficult to be certain whether the maximal decreases in tissue volume at the highest stimulation frequencies are different. But in those preparations where satisfactory records were obtained for both nerves the change in tissue volume with deep fibular nerve stimulation always exceeded the change due to tibial nerve stimulation. This relationship would further support tibial nerve tissue volume change by venous constriction.

In summary, the deep fibular nerve does increase precapillary resistance by arteriolar constriction but does not produce a proportionate active constriction of the venules, thereby allowing reduced capillary pressure. However, tibial nerve stimulation causes only moderate increases in precapillary resistance, but also causes active constriction of the veins, thereby causing increased postcapillary resistance which maintains capillary pressure.

The authors express appreciation to William Cook for technical assistance. This project was supported by a grant from American Heart Association, Florida Affiliate.

1. Zimmerman BG. Separation of responses of arteries and veins to sympathetic stimulation. Circ Res **18:** 429–436, 1966.
2. Zimmerman BG. Sympathetic vasodilation in the dog's paw. J Pharmacol Exp Ther **152:**81–87, 1966.
3. Davis DL, Hammond MC. Blood flow redistribution in the dog paw. Amer J Physiol **215:**496–501, 1968.
4. Hammond MC, Davis DL, Dow P. Segmental responses of dog paw vasculature. Circ Res **23:**1–9, 1968.
5. Davis DL. Effect of sympathetic stimulation on dog paw volume. Amer J Physiol **205:**989–994, 1963.
6. Baker CH, Davis DL, Sutton ET. Control of A-V shunt and capillary circuits in the dog hindpaw. Proc Soc Exp Biol Med **157:**536–540, 1978.
7. Baker CH, Davis DL, Sutton ET. Frequency dependent control of nutritional and non-nutritional circuits in the dog paw. Blood Vessels **16:**87–97, 1979.
8. Baker CH, Davis DL, Sutton ET. Neural control of nutritional and non-nutritional circuits in the dog hindpaw. Amer J Physiol **234:**H384–H391, 1978.
9. Baker CH, Davis DL, Lindsey BG. Innervation sites

of dog hindpaw series and parallel vascular circuits. Blood Vessels **20**:135–144, 1983.
10. Baker, CH. Fibrinogen-I^{131}, T-1824 and red cell-Cr^{51} spaces following hemorrhage. Amer J Physiol **205**: 527–532, 1963.
11. Hamilton WF, Moore JW, Kinsman JM, Spurling RG. Simultaneous determination of the pulmonary and systemic circulation time in man and a figure related to the cardiac output. Amer J Physiol **84**:338–344, 1928.
12. Baker CH. Blood flow and volume redistribution with elevated venous pressure. Amer J Physiol **218**:674–680, 1970.
13. Baker CH. Blood flow and volume redistribution with A-V shunt occlusion. Amer J Physiol **223**:1365–1370, 1972.
14. Davis DL, Baker CH. Comparison of changes in blood volume and opacity in dog digital pad and tongue. J Appl Physiol **27**:613–618, 1969.
15. Baker CH, Menninger RP, Schoen RE, Sutton ET. Skeletal muscle vascular volume changes with increased venous pressure. Blood Vessels **13**:222–237, 1976.

Received May 21, 1985. P.S.E.B.M. 1986, Vol. 181.
Accepted November 1, 1985.