TRANSVASCULAR FLUID MOVEMENT AND SEGMENTAL VASCULAR RESISTANCES IN RESPONSE TO ENDOTOXIN SHOCK

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By

W. Jeffrey Weidner

AN ABSTRACT OF A THESIS

Submitted to
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ABSTRACT

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Collateral-free, innervated, naturally perfused forelimbs were used to study transcapillary fluid fluxes in skin and skeletal muscle in pentobarbitalized dogs (N=20) subjected to endotoxin shock. Transcapillary fluid fluxes were estimated from changes in forelimb weight and segmental vascular resistances (large artery, small vessel, large vein). E. coli endotoxin (2 mg/kg or 5 mg/kg) produced sustained decreases in forelimb skin and skeletal muscle vascular pressures and blood flows; segmental vascular resistances increased, especially in skin. Forelimb weight decreased throughout a 4 hour period. The initial rapid weight loss (0-10 min) is largely attributable to a decreased vascular volume subsequent to constriction of forelimb capacitance vessels i.e. small vessels and large veins. The slow weight loss (10-240 min) was associated with further resistance increases in skin capacitance vessels and decreases toward control in skeletal muscle capacitance vessels; the net effect being a fall in total forelimb vascular resistance toward control. This

suggests and increasing forelimb vascular volume from minutes 10-240 and, hence, that the weight loss over this period is attributable to extravascular water reabsorption from the interstitial and/or intracellular compartments. All vascular pressures including small vein pressure, which represents a minimum for Pc, were decreased in skin and skeletal muscle (0-240 min) suggesting that net interstitial water influx may have occurred subsequent to a fall in Pc. A fall in Pc would occur if the decrease in aortic pressure and the increase in precapillary resistance overwhelmed the effect of the increase in postcapillary resistance. Arterial plasma osmolarity increased; this could promote either intracellular hydration or dehydration depending on the origin of the extra osmotically active particles. These data fail to support the contention that extensive net fluid efflux into skin and skeletal muscle is a determinant of irreversibility in endotoxin shock.

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CHAPTER I

INTRODUCTION

Endotoxin shock is clinically associated with a high mortality rate and a serious obstacle to the successful treatment of this condition is the lack of complete data on its pathophysiology. This study is an attempt to provide further definitive information on one aspect of endotoxin shock, i.e. the role of transcapillary fluid fluxes in skin and skeletal muscle in determining irreversibility.

Transvascular fluid efflux, especially into skeletal muscle, has been suggested as a possible determinant of irreversibility in endotoxin shock. The literature on this subject, however, is inconsistent. Several factors may account for these conflicting observations: species variation in response to endotoxin; error associated with plasma volume measurements made with dilutional techniques, particularly in vasoconstricted states; the inability of dilutional techniques to separate measured decreases in blood volume due to fluid efflux from decreases due to intravascular pooling. Moreover, transcapillary fluid fluxes in skeletal muscle have not been studied specifically, but the alleged fluid efflux has been inferred from whole body changes in hematocrit and

plasma volume. In an attempt to circumvent some of these problems, endotoxin shock was studied utilizing a gravimetric technique. Transcapillary fluid fluxes in the canine forelimb were estimated from changes in forelimb weight and segmental vascular resistances. The forelimb was selected as the test organ since it is largely composed of skin and skeletal muscle.

CHAPTER II

REVIEW OF LITERATURE

Endotoxin shock, often called bacteremic or septic shock, is a condition resulting from the liberation of a lipoprotein-carbohydrate complex from the cell wall of a certain gram negative bacteria, of which E. coli is an example. Endotoxin, once it enters the vascular system of an animal, has pronounced deleterious effects and is often times fatal. Although the response to endotoxin is not the same in all species, hypotension is common to all.

Canine endotoxin shock following i.v. injection of purified endotoxin is generally associated with the following responses (1,28,45,48,50): systemic arterial pressure (SAP) falls abruptly within 2-5 minutes, transiently recovers toward control levels (min 20-45), and then gradually falls unto death; right atrial pressure (RAP) falls within 1-3 minutes and remains below control; cardiac output (CO) falls precipitously within 2-5 minutes and follows a pattern similar to SAP; calculated plasma volume (PV) has been reported to progressively decrease up to 36%; after an initial bradycardia, heart rate remains elevated above control; no significant change in myocardial contractility occurs until the

terminal stages. Total peripheral resistance (TPR) increases markedly within 2-5 minutes following endotoxin administration, wanes (min 20-45), and then either increases from 60 minutes until death or further decreases (until min 120-180) before increasing until death. Hematocrit, after an initial decrease (0-10 min), has been reported to rise continuously.

Decreased flow, subsequent to a decreased blood volume resulting from vascular pooling and/or from transvascular fluid loss, is thought to be the major determinant of irreversible shock from endotoxin in the dog (1,22,30,50,51). Endotoxin administration into live animals elicits a number of responses which are known to affect transcapillary water movement across capillaries and the intravascular distribution of blood. Transcapillary water movement is regulated by the transmural hydrostatic pressure gradient and by the transmural colloid osmotic pressure gradient. Capillary hydrostatic pressure (Pc) is an important determinant of the former; a rise in this variable above colloid osmotic pressure (COP) promotes fluid efflux, while a fall in Pc below COP facilitates fluid influx. Pc is determined by the vessel wall compliance and capillary blood volume. Capillary blood volume is determined by the pre-to-postcapillary resistance ratio and by aortic pressure and RAP. Resistance in the pre-and postcapillary segments is related to vessel caliber. Vessel caliber is a function of changes in vascular smooth muscle (active changes) and changes in transmural pressure independent of changes in smooth muscle activity (passive changes). A

decrease in SAP, RAP, or postcapillary resistance, or increase in precapillary resistance will lower Pc. Likewise, an increase in SAP, RAP, or postcapillary resistance, or a decrease in precapillary resistance will produce the opposite effect on Pc. The pre-to-postcapillary resistance ratio can also be affected by changes in the viscosity of the blood, if such changes are of significant magnitude and differentially affect the precapillary and postcapillary vessels. The transmural colloid osmotic pressure gradient can be altered by a change in microvascular permeability to plasma proteins, by changes in concentrations of osmotically active particles, as well as by abnormal lymph drainage.

Endotoxin shock is associated with altered net fluid fluxes across the capillaries. Net fluid influx is thought to occur initially. Measurements of hematocrit and plasma protein concentration are compatible with the early influx of fluid in that both decrease initially in the intact dog (1,2,45). In splenectomized dogs similar changes are seen in hematocrit and plasma protein concentration; also PV reportedly increases (1,2,52). In monkeys PV increases; hematocrit and plasma protein concentration decrease (1,18,50). This also suggests that the early stage of endotoxin shock is associated with extravascular fluid reabsorption. The initial fluid influx following endotoxin injection has been attributed to a fall in Pc, especially in skeletal muscle. Pc is thought to fall subsequent to the fall in SAP and RAP and an increased pre-to-postcapillary resistance ratio

(6,16,18,23,30). The fluid influx is a compensatory mechanism which results from sympathoadrenal discharge subsequent to arterial hypotension. This mechanism serves to increase blood volume.

The direction of net fluid movement has been reported to reverse with time (45-60 minutes after endotoxin administration) due to increased Pc and/or increased microvascular permeability to proteins (1,2,29,33). This is viewed as important in the development of irreversibility since it serves to critically reduce the effective blood volume. In dogs a continuous rise in hematocrit and plasma protein concentration has been observed after the initial decrease (1,2,47). Weight continuously increases in forelimbs perfused at a constant flow. This has been interpreted as evidence for a net fluid efflux (30,44). However, this only means that the veins constrict. And, although compatible with PV loss, does not necessarily mean PV loss occurs at natural flow. Dogs also develop diarrhea, which demonstrates that some fluid is lost into the intestinal lumen. The volume of fluid lost through the intestine is relatively small and is not enough in itself to cause irreversibility (17). This represents species variability for intestinal necrosis and does not occur in cats or primates (24,32,52).

The intravascular distribution of blood is affected by factors that affect vascular capacity. Vascular capacity is affected by active and passive changes in vessel caliber. Reported losses in PV in both the early and late stages of

endotoxin shock are frequently attributed to extensive fluid efflux. These losses, however, may be due wholly or in part, to vascular pooling of blood. Peculiar to the canine species during the early stage of endotoxin shock is an hepatic venoconstriction occurring with 1-2 minutes after administering endotoxin. This venoconstriction decreases in intensity at 3 minutes and essentially disappears within 20 minutes. It is responsible for the initial rise in Pc in the hepatosplanchnic beds, and has been implicated as the cause of impeded venous return which serves to precipitate the early hypotensive stage of endotoxin shock in the dog (1,21,24,26, 31,34,36,47). This venoconstriction is reported to be responsible for vascular pooling of blood in the hepatosplanchnic beds during the early phase of endotoxin shock (9,17,26,34). Increases of up to 35g/100g tissue weight occur in the small intestine (17). Liver weight increases from 80 to 350g have been reported to occur within 3 minutes following endotoxin. This response is short lived however, as weight returns to control within 30 minutes (34). In the late phase of endotoxin shock little or no evidence exists for vascular pooling in the hepatosplanchnic beds or other organs.

During endotoxin shock, SAP and RAP decrease appreciably while peripheral precapillary resistance increases. This would tend to lower Pc. In order for Pc to increase under these conditions, the rise in postcapillary resistance would have to overcome the effects of the fall in SAP and RAP and

the increase in precapillary resistance in order to raise Pc above colloid osmotic pressure and promote a fluid efflux. This seems unlikely considering the hemodynamics of the shock state. After endotoxin administration SAP frequently falls to 30-40 mm Hg and RAP is frequently 0 mm Hg or less. In this case, if the pre-to-postcapillary resistance ratio fell from a control of 4 to as low as 1, Pc would remain between 15 and 20 mm Hg, well below COP. It is thus unlikely that extravasation of fluid takes place during extreme hypotension unless capillary membrane permeability to plasma proteins is substantially increased.

Evidence exists which suggests that capillary membrane permeability to plasma proteins is increased during endotoxin shock (1,44). A fall in the transmural colloid osmotic pressure gradient could promote fluid efflux even if Pc does not increase. If Pc falls, however, a decreased transmural colloid osmotic pressure gradient would not necessarily result in extravasation of fluid. Indeed, if the transmural hydrostatic pressure gradient fell proportionately more than the transmural colloid osmotic pressure gradient, filtration of fluid would not occur, but rather extravascular fluid reabsorption from tissue would result. The reported decrease in measured blood volume is usually attributed to intravascular fluid loss by filtration, although it could as well be attributed to intravascular pooling of blood since, in vasoconstricted states, the indicator may not penetrate into the pooled blood. In splenectomized dogs dilutionaly measured

PV does not significantly change or increases slightly; hematocrit and plasma protein concentration decrease suggesting hemodilution rather than fluid loss (1,2,48). In primates including man, there is likewise no evidence for a progressive PV loss, measured PV does not progressively decrease, nor do hematocrit or plasma protein concentrations progressively increase (5,9,22,30,52). Weight has been reported to continuously decrease for 120 minutes in response to endotoxin (1 mg/kg) in autoperfused canine forelimbs (29). In view of these observations it is felt that the contention that extensive transvascular fluid loss by filtration as being an important determinant of irreversibility in endotoxin shock should be reexamined.

Following systemic administration of endotoxin TPR has been reported to increase greatly within 5 minutes, wane from 20-45 minutes, and either increase from 60 minutes until death or continue to further decrease toward control for a variable period and then increase until death (1,31,35). Systemic administration of endotoxin has been reported to produce an early transitory phase of increased total resistances in autoperfused splanchnic, hepatic, renal, hindlimb and forelimb vascular beds and then resistances partially wane with time (20,26,35,36). The cerebral and coronary vascular beds show relatively little change in resistance in response to systemically administered endotoxin (35,43). Systemic infusion of endotoxin also increases resistance in vascular segments which are series coupled in the autoperfused

forelimb. Segmental vascular resistances (large artery, small vessel, large vein) increase abruptly within 2-5 minutes and then partially wane with time (29). In the study just cited, however, the resistance calculations are not skin, skeletal muscle, or total forelimb values. Skin segmental pressure gradients were divided by total forelimb flow (brachial vein outflow plus cephalic vein outflow).

Local I.A. administration of endotoxin to various vascular beds has been reported to have only small transient effects on the renal, hindlimb and forelimb vascular resistances (10,19,30,35). The coronary bed has been reported to show a decreased resistance when given endotoxin locally (8). However, the isolated liver and small intestine when perfused with endotoxin show increased resistances (26).

The observed change in TPR in canine endotoxemia is seemingly a phenomenon due both to active and passive factors. Active changes in vessel caliber are due to indirect actions of endotoxin, since local administration apparently has little or no effect on most vascular resistances (45). Indirect active changes in vessel caliber are due to neurogenic effects and to chemical factors which are liberated into the bloodstream by endotoxin (38,39,40). Passive changes in vessel caliber may be precipitated by blood viscosity changes which affect vascular resistance and hence blood flow. These may be precipitated either directly or indirectly by endotoxin, i.e., increased hematocrit and hypercoaguability (15). TPR may also rise as the result of passive geometrical factors,

such as decreases in vessel radius which occur as a result of a fall in transmural pressure, especially in the late stages of endotoxin shock (19). It has been shown that hepatosplanchnic resistance changes and pooling are not dependent on an intact nerve supply to the viscera or to the presence of the adrenal glands (18). Late changes in resistance are more pronounced in normal dogs than splenectomized dogs and can be related to a rise in blood viscosity resulting from splenic emptying (1,2,16). Monkeys, in response to endotoxin, show a decreased TPR throughout the experimental period, as do eviscerated dogs (9,22,25).

The development of a progressive systemic hypotension in the dog following endotoxin administration has been explained by decreases in CO (9,23). Endotoxin has been reported to cause a lowered "setting" of the baroreceptors which would result in a lowering of the regulated systemic pressure (49). Since heart rate is slightly elevated after an initial bradycardia and cardiac strength is reportedly unimpaired in response to endotoxin, the decreased CO is reportedly due to an impeded venous return (2). The alleged fluid efflux would contribute to the decreased venous return. Certain blood borne substances such as serotonin, catecholamines, and histamine, have been implicated as causing an hepatic venoconstriction. This, combined with decreases in responsiveness to pressor agents of peripheral precapillary vessels and the action of vasodilator metabolites, has been reported to facilitate fluid efflux and to participate in decreasing

venous return and in a transient waning of TPR (1,2,30,48,51). These effects, especially the increased metabolites, are said to be the result of stagnant anoxia (1,2).

In conclusion, transcapillary fluid loss has been implicated as an important determinant in the genesis of irreversibility in canine endotoxin shock, particularly into skeletal muscle. Fluid loss into muscle could be potentially important since this tissue comprises about 65% of total body weight. If filtration is large enough to cause irreversibility, fluid loss into muscle would be very important. Certain inconsistencies exist in the literature surrounding the genesis of irreversibility in endotoxin shock with regard to fluid efflux. Therefore a reexamination of the vascular responses of skin and skeletal muscle was undertaken. We attempted to measure transcapillary fluid fluxes in skin and skeletal muscle utilizing a technique which does not use dye dilutional methods. Continuous recordings of forelimb weight and calculations of forelimb skin, skeletal muscle and total vascular resistances in both precapillary and postcapillary vascular segments were made in an effort to determine the direction and mechanism of transvascular fluid fluxes.

CHAPTER III

METHODS

Forelimb Experiments:

Dogs of either sex having an average weight of 18 kg were anesthetized with sodium pentobarbital (30-35 mg/kg) and allowed to breathe spontaneously through a cuffed endotracheal tube. Skin of the right forelimb was circumferentially sectioned 3-5 cm above the elbow. The right brachial artery, forelimb nerves, and brachial and cephalic veins were isolated, and the muscles and remaining connective tissue sectioned by electrocautery. The humerous was cut and the ends of the marrow cavities packed with bone wax. Blood entered the limb only through the brachial artery and exited only through the brachial and cephalic veins. The forelimb nerves (median, ulnar, radial and musculocutaneous) were left intact and coated with an inert silicone spray to prevent drying. Heparin was administered in an initial dose of 10 mg/kg and hourly supplements of 2.5 mg/kg. The following cannulations were made to obtain segmental pressure gradients in skin and skeletal muscle: 1) skin small artery from the third superficial volar metacarpal artery on the underside of

the paw; 2) muscle small artery from a vessel supplying a flexor muscle in the upper portion of the forelimb; 3) skin small vein from the second superficial dorsal metacarpal vein on the upper surface of the paw; 4) muscle small vein from one of the deep vessels draining a flexor muscle in the middle portion of the forelimb; 5) skin large vein from the cephalic vein via a side branch at the level of the elbow; and 6) muscle large vein from the brachial vein via a side branch at the level of the elbow. All cannulas were small bore polyethylene tubes (PE-10 to PE 60) and cannulation was accomplished using the wedge pressure technique. With this technique the cannulated small vessel acts as an extension of the catheter and, hence, the pressure measured is that in the collateral vessels joined by the cannulated vessel. The measured pressure is a lateral pressure as long as the cannulated vessel is patent and without valves (this is verified by the ability to freely withdraw blood from and to flush into the vessel). The presence of the catheter does not measurably alter the pressure because, in the forelimb, the cannulated vessel is a negligible fraction of the total cross-sectional area of the vascular bed and there are many artery to artery and vein to vein anastomoses (12,13,37). Pressures were measured with low volume displacement Statham transducers (P23G-B) and recorded on a Hewlett-Packard direct-writing oscillograph.

Brachial and cephalic veins were partially transsected 3-5 cm downstream from the site of the large-vein pressure

measurement and the distal end of each vessel was catheterized with a short section of PE-320 tubing. Total venous outflow was directed into a reservoir maintained at constant volume by a pump which returned blood to the left jugular vein. Blood flow was determined by timed collections of the two venous outflows. In this preparation the median cubital vein represents the major anastamotic channel between brachial and cephalic veins. This vessel was ligated and used for large vein pressure measurements, one catheter directed to the cephalic vein, the other to the brachial vein. Thus brachial venous flow was predominantly from skeletal muscle while cephalic flow was predominantly from skin. Although this approach may not completely isolate the blood flow through skin from that through muscle, flow separation is sufficient to compare resistance changes in the two parallel-coupled beds (3,11,41).

After cannulation the limb was placed on a wire mesh platform attached to a strain-gauge balance (46). In all experiments the balance was calibrated by the addition of a 1g weight which produced a 10 to 20 mm pen deflection on the oscillograph. Calibrations were made before each flow measurement. Mean systemic arterial pressure was continuously monitored from a catheter (PE-240) in the left carotid artery. Following a short control period either 20 cc of saline or purified E. coli endotoxin (Difco Laboratories,Detroit, Mich.) suspended in 20 cc of normal saline, was infused into a cannulated jugular vein by means of a Harvard constant infusion

pump. Infusion time was 10 minutes. The total dose was either 2 mg/kg or 5 mg/kg. Limb weight was continuously monitored and all pressures and flows were determined twice during a preinfusion control period and at the 2nd, 5th, 10th and 15th minute after onset of infusion. Subsequently, pressures and flows were measured every 15 minutes throughout a 4 hour experimental period.

Total and segmental vascular resistances (large artery, small vessel, large vein) in muscle and skin were calculated by dividing brachial or cephalic flow into the corresponding pressure gradient. In addition, resistances in the total forelimb and in each of the combined skin and muscle segments were calculated as (11,41):

$$\text{Total forelimb resistance} = \frac{R_{ts} \cdot R_{tm}}{R_{ts} + R_{tm}} \quad (1)$$

$$\text{Total forelimb large artery resistance} = \frac{R_{sa} \cdot R_{ma}}{R_{sa} + R_{ma}} \quad (2)$$

$$\text{Total forelimb small vessel resistance} = \frac{R(s\text{-}v)s \cdot R(s\text{-}v)m}{R(s\text{-}v)s + R(s\text{-}v)m} \quad (3)$$

$$\text{Total forelimb large vein resistance} = \frac{R_{sv} \cdot R_{mv}}{R_{sv} + R_{mv}} \quad (4)$$

where: R = resistance in mm Hg X min^{-1} X ml^{-1} X $100g^{-1}$; t = total; s = skin; m = skeletal muscle; a = large artery; s-v = small vessel; v = large vein. Mean transmural pressure in each segment was obtained by: $P_1 + P_2/2$ where: P_1 = inflow pressure; P_2 = outflow pressure.

Systemic arterial plasma concentrations of Na^+, K^+ Ca^{++} and Mg^{++}, as well as Hmct, pH, and plasma osmolality were determined from 10 ml samples of carotid arterial blood drawn during the control period and at hourly intervals during the experimental period. Na^+ and K^+ were measured with a flame photometer, Ca^{++} and Mg^{++} by atomic absorption. Osmolality was obtained by the freezing point depression method (Advanced osmometer). PH was measured with the Radiometer pH meter. Hematocrits were determined in triplicate by the microcapillary technique.

Gracilis Muscle Experiments:

Five dogs having an average weight of 18 kg were prepared for surgery in the manner described in the forelimb studies. The right gracilis muscle was ligated at both musculotendinous junctions and all blood vessels except the gracilis artery and vein were tied. A side branch of the right gracilis vein was cannulated in order to record venous pressure. SAP was monitored by means of a catheter inserted into the left femoral vein, ligated such that its only inflow was from the gracilis vein, was cannulated in order to collect venous outflow from the gracilis muscle. Blood was collected in a reservoir held at constant volume with a pump which returned blood to the animal via the jugular vein. Flow was measured by timed collections of the venous outflow. Vascular resistance was calculated by dividing the pressure gradient (mm Hg) between measured SAP and the gracilis vein by gracilis blood flow (ml/min). Saline or endotoxin (5 mg/kg) was infused as

previously described. All parameters were measured twice during a control period, 2, 5, 10, and 15 minutes after initiating the infusion and every 15 minutes thereafter throughout the remainder of a 4 hour period.

CHAPTER IV

RESULTS

Data presented herein was obtained from 20 dogs. Ten were used for each dose level. Six additional dogs died in response to 5 mg/kg endotoxin and two additional dogs died in response to 2 mg/kg endotoxin prior to the end of the experimental period. Data from these 8 animals are not presented. All data are presented as the mean value or the mean value $\pm$ standard error. The unpaired student t test was used to determine statistical probability of difference between data.

Forelimb weight - 2 mg/kg and 5 mg/kg endotoxin (Figure 1).

The legs were isogravimetric prior to the infusion. Infusion consistently produced an initial rapid weight loss (0-10 min), 6.5 $\pm$ 0.8g in response to the low dose and 8.5 $\pm$ 2.7g in response to the high dose of endotoxin. This was followed by a more gradual weight loss resulting in a total mean loss of 12.5 $\pm$ 3.7g by min 240 in response to the low dose and a loss totaling 21.7 $\pm$ 3.8g at min 240 in response to the high dose. Both dose levels of endotoxin produced responses which followed a similar pattern, but the higher dose tended to produce greater effects.

Vascular pressures - 2 mg/kg and 5 mg/kg endotoxin (Figures 1, 3 and 5).

Control SAP in the 5 mg/kg group was 131 ± 5.2 mm Hg, fell 45-50 mm Hg below control by 10 minutes and was 59 ± 7.0 mm Hg at min 240. SAP at min 0 was 130 ± 5.0 in the 2 mg/kg group, fell to a level 40-45 mm Hg below control and was 78 ± 8.4 mm Hg at min 240. The pattern of the fall in small artery pressures was similar to the fall in SAP in response to either dose. Venous pressures fell and remained below control throughout the 4 hour period. The pressure decreases were usually more marked in response to the high dose of endotoxin. Both doses produced changes in transmural pressure which paralleled those changes seen in SAP (see appendix). Greatest percent change occurred in venous segments.

Blood flow - 2 mg/kg and 5 mg/kg endotoxin (Figures 1, 3 and 5).

Total blood flow fell markedly in response to either dose (from 21.7 ± 1.9 ml/min/100g forelimb to 3.9 ± 0.8 by min 10 and to 3.0 ± 0.6 by min 240 in response to 5 mg/kg endotoxin and from 26.0 ± 2.7 to 5.2 ± 0.6 in response to 2 mg/kg endotoxin). The effect on blood flow was most marked in response to the high dose. Skin and muscle blood flows fell in response to either dose and were maintained at low levels throughout the 4 hour period, although flow through muscle recovered to a higher level than skin blood flow.

Total vascular resistances - 2 mg/kg endotoxin (Figures 2, 4 and 6).

Total vascular resistance (mm Hg/min/ml/100g forelimb) increased from a mean control value of 5.9 ± 0.9 to a maximum of 19.4 ± 4.1 at 10 min, and fell to 14.0 ± 1.3 by min 240. Total skin resistance increased from a mean control value of 12.8 ± 3.0 to 130.4 ± 30.0 by min 240. Total muscle resistance increased from a mean control of 12.8 ± 1.3 to 55.2 ± 7.0 at 10 min and then fell to 28.3 ± 4.4 at min 240.

Total vascular resistances - 5 mg/kg endotoxin (Figures 2, 4 and 6).

Total forelimb resistance (mm Hg/min/ml/100g forelimb) increased from a mean control value of 6.1 ± 0.5 to 36.5 ± 9.3 at 10 min and oscillated between 16.1 and 25.6 for the duration of the experiment. Total skin resistance was 12.5 ± 1.8 at control, increased to 65.8 ± 17.4 at 10 min and to 131.6 ± 28.6 by min 240. Total muscle resistance was 13.1 ± 1.0 at control, increased to a maximum of 94.1 ± 21.2 at 10 min and decreased to 35.7 ± 4.2 at min 240.

Segmental vascular resistances - 2 mg/kg endotoxin (Figures 2, 4 and 6).

Calculated resistances (mm Hg/min/ml/100g forelimb) in the three cutaneous vascular segments were maximal during the last hour of the experimental period and showed a proportionately greater rise than did the corresponding muscle segmental vascular resistances. Muscle small vessel and large vein vascular resistances were maximal at 10 min after which time

resistance gradually decreased toward control. Muscle large artery resistance increased to maximal by the end of the 4 hour period. Total arterial resistance increased to a maximum by min 240, while total small vessel and total venous resistances reached maxima by min 10 and then decreased. Both the large artery resistance to large vein resistance ratio and the prevenous resistance to venous resistance ratio were unchanged compared to control in skin. But both resistance ratios increased in muscle during the latter part of the experimental period (see Appendix table 2 and 3).

Segmental vascular resistances - 5 mg/kg endotoxin (Figures 2, 4 and 6).

Segmental vascular resistance increased proportionately more in skin than in muscle. Calculated resistances (mm Hg/min/ml/100g forelimb) in the three cutaneous segments continued to increase throughout the experimental period. In muscle large artery, small vessel and large vein vascular resistance increased to maximum values at 10-15 minutes and subsequently declined toward control. Total small vessel and total venous resistances reached maximum values by min 10 and decreased, while total arterial resistance was maximum at min 240. The large artery resistance to large vein resistance ratio was unchanged relative to control in both skin and muscle. The prevenous resistance to venous resistance ratio in both muscle and skin was also unchanged relative to control (see Appendix table 2 and 3).

Blood chemistry (Table 1).

The effects of 2 mg/kg and 5 mg/kg endotoxin on blood chemistry are presented in Table 1. Both dose levels of endotoxin produced increases in hematocrit, osmolarity, $[K^+]$, and $[H^+]$. No significant change was seen in plasma $[Ca^{++}]$or $[Mg^{++}]$.

Control experiments (Table 2).

In saline infused animals studied for 4 hours, forelimb weight and systemic pressure were unchanged relative to control throughout the entire observation period. Total skin and skeletal muscle vascular resistances increased slightly but significantly during this time; the resistance increases however, were largely confined to the small vessel segment. Hence, the marked changes in forelimb weight and systemic pressure which occurred in following infusion of endotoxin must largely be attributed to its effects and not to spontaneous changes occurring with time. The spontaneous resistance increase in skin in the saline infused animals could only account for a small fraction of the increased total skin resistance in the animals given endotoxin. In muscle, however, the spontaneous resistance increase in saline infused animals could account for a significant fraction (approximately 45% at min 240) of the resistance increase in the animals administered endotoxin.

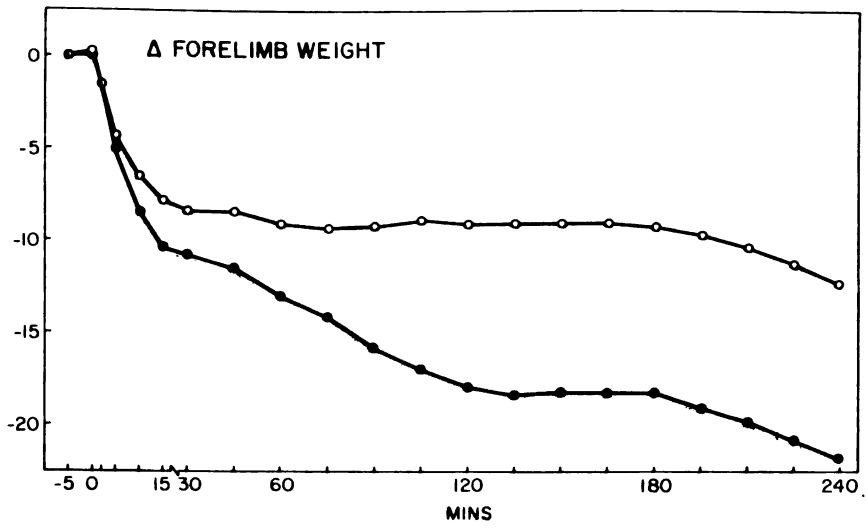
Gracilis muscle experiments (Table 3).

Five mg/kg endotoxin produced significant decreases in SAP, gracilis vein pressure, and gracilis blood flow.

Gracilis vascular resistance increased (0-10 min) and then decreased toward control levels to min 240. In saline animals SAP decreased from 142.3 ± 2.3 mm Hg at control to 121.7 ± 3.4 at min 240; gracilis vascular resistance increased slightly but significantly with time; gracilis blood flow fell from 4.1 ± 1.2 ml/min/100g tissue at control to 2.7 ± 1.3 at min 240; and gracilis vein pressure remained relatively steady throughout the experimental period.

Figure 1. Effects of endotoxin 2 mg/kg (open circles, N=10) and 5 mg/kg (closed circles, N=10) on forelimb weight (g), total forelimb blood flow (ml/min/100g forelimb), and systemic pressure (mm Hg).

FORELIMB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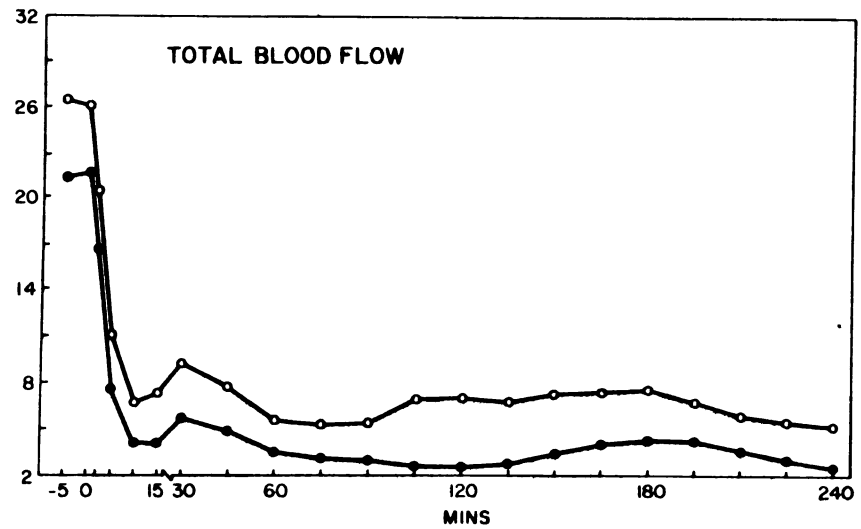
MINS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26
20
14
8
2
-5 0
15 30
60
120
180
240
MINS

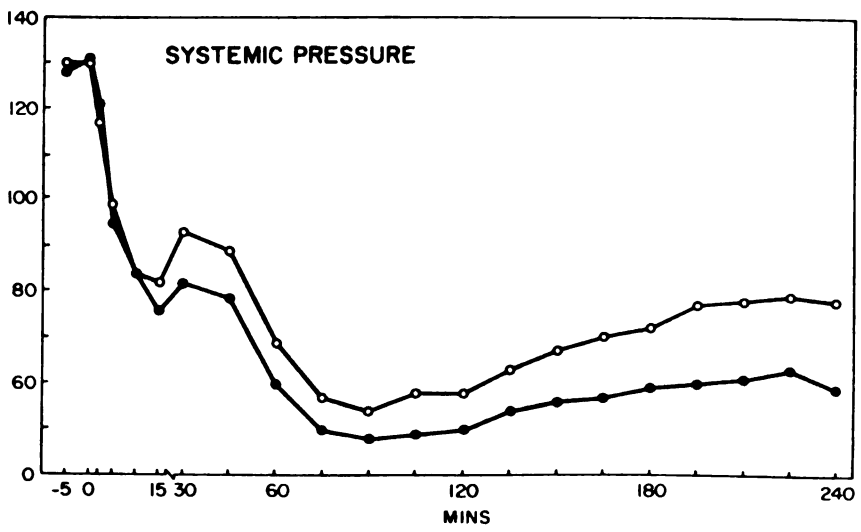
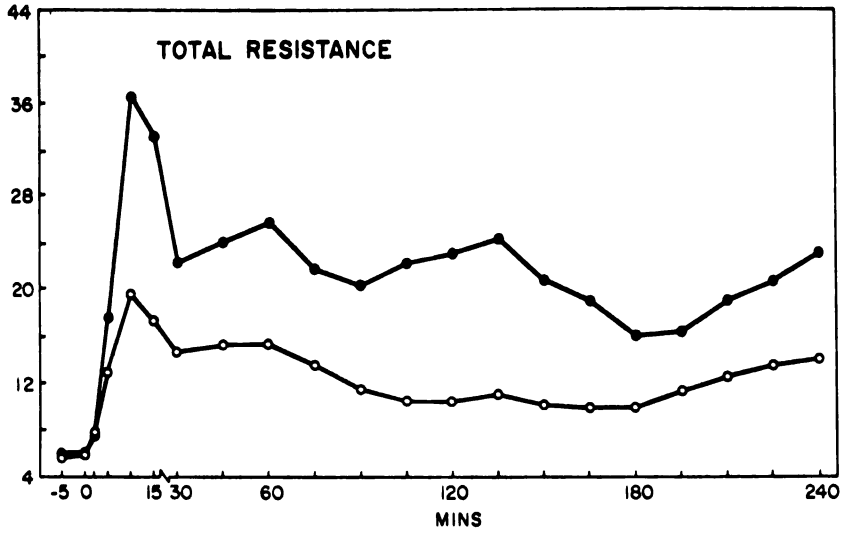

SYSTEMIC PRESSURE
140
120
100
80
60
0
-5 0
15 30
60
120
180
240
MINS

Figure 2. Effects of 2 mg/kg and 5 mg/kg endotoxin on total and segmental vascular resistances (mm Hg/ml/min/ 100g forelimb). Symbols and N values correspond to those in Figure 1.

FORELIMB


TOTAL RESISTANCE
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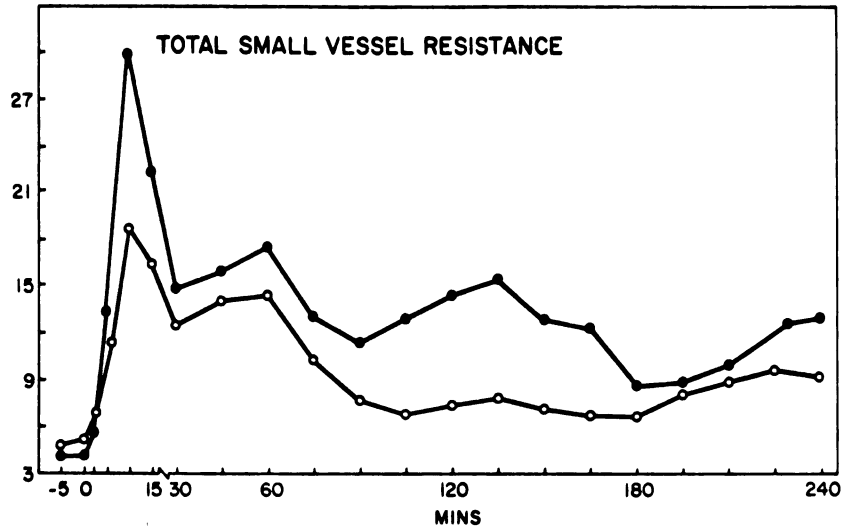
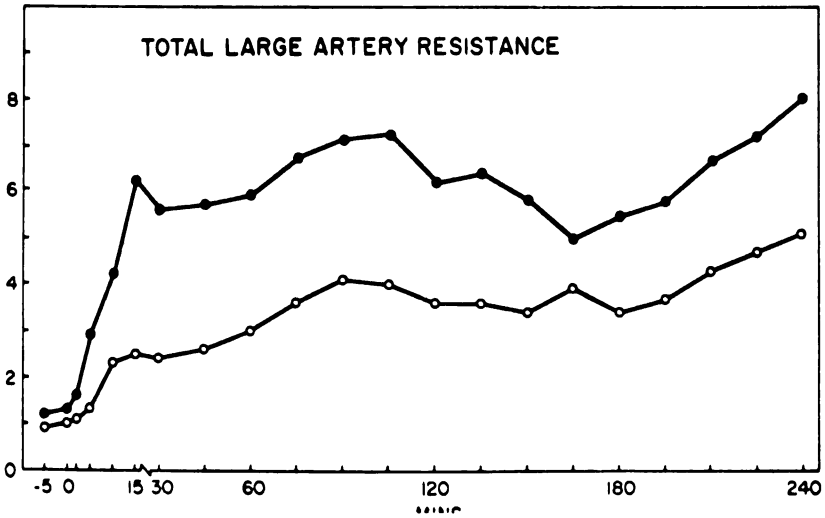
36
28
20
12
4
-5 0
15 30
60
120
180
240
MINS


TOTAL SMALL VESSEL RESISTANCE
27
21
15
9
3
-5 0
15 30
60
120
180
240
MINS


TOTAL LARGE ARTERY RESISTANCE
8
6
4
2
0
-5 0
15 30
60
120
180
240

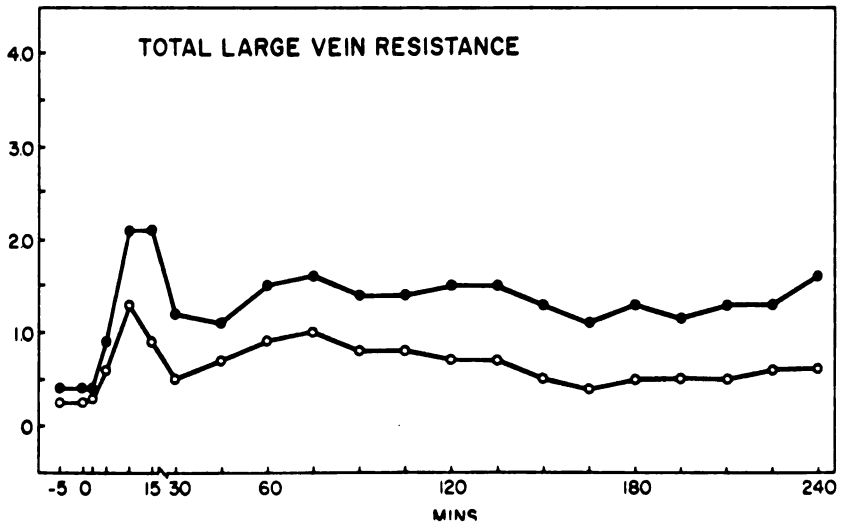
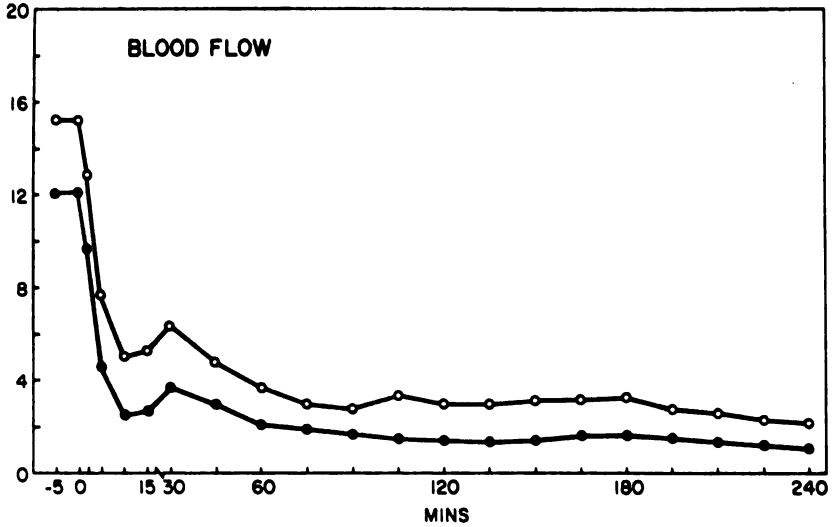
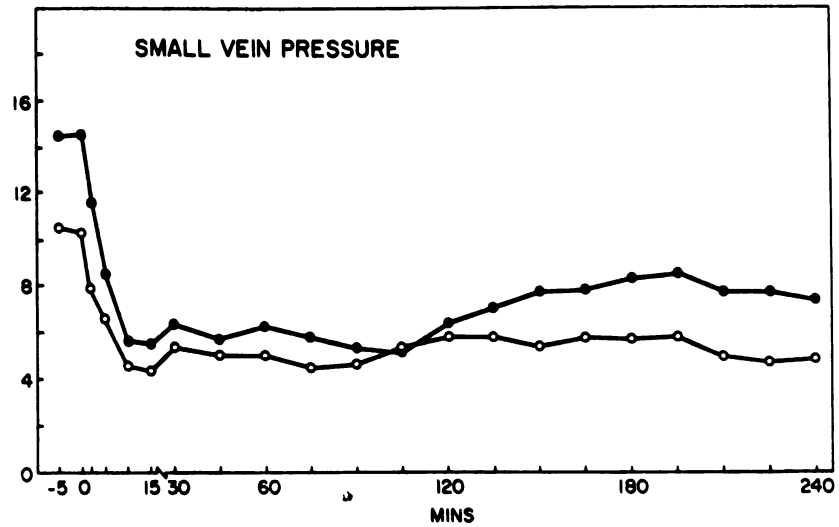
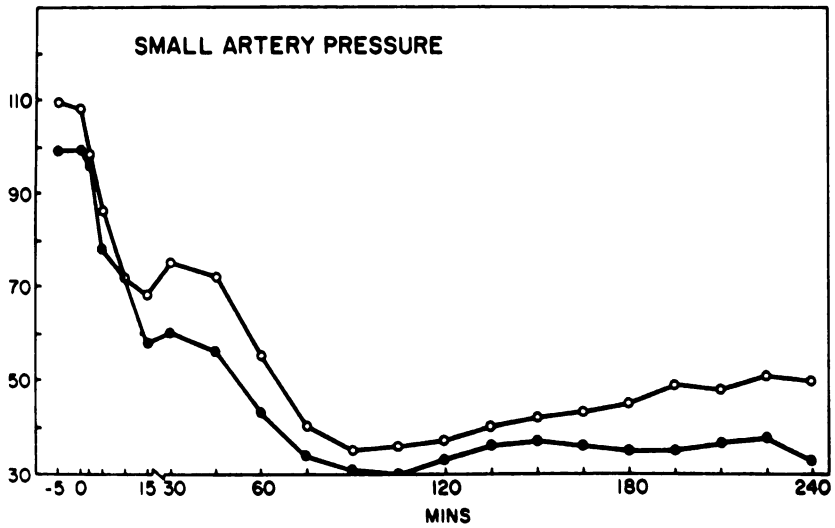

TOTAL LARGE VEIN RESISTANCE
4.0
3.0
2.0
1.0
0
-5 0
15 30
60
120
180
240
MINS

Figure 3. Effects of 2 mg/kg and 5 mg/kg endotoxin on blood flow (ml/min/100g forelimb), and large and small vessel pressures (mm Hg) in skin vasculature of dog forelimb. Symbols and N values correspond to those in Figure 1.

SKIN


BLOOD FLOW
20
16
12
8
4
0
-5 0
15 30
60
120
180
240
MINS


SMALL VEIN PRESSURE
16
12
8
4
0
-5 0
15 30
60
120
180
240
MINS


SMALL ARTERY PRESSURE
110
90
70
50
30
-5 0
15 30
60
120
180
240
MINS

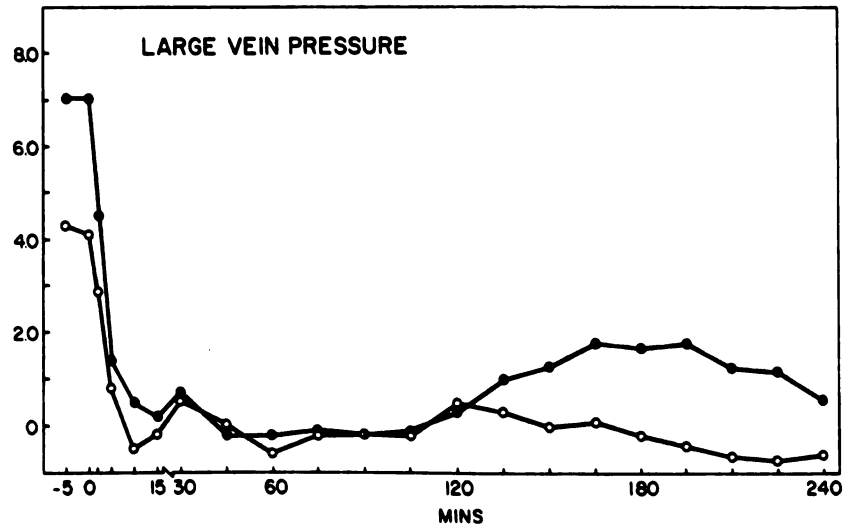
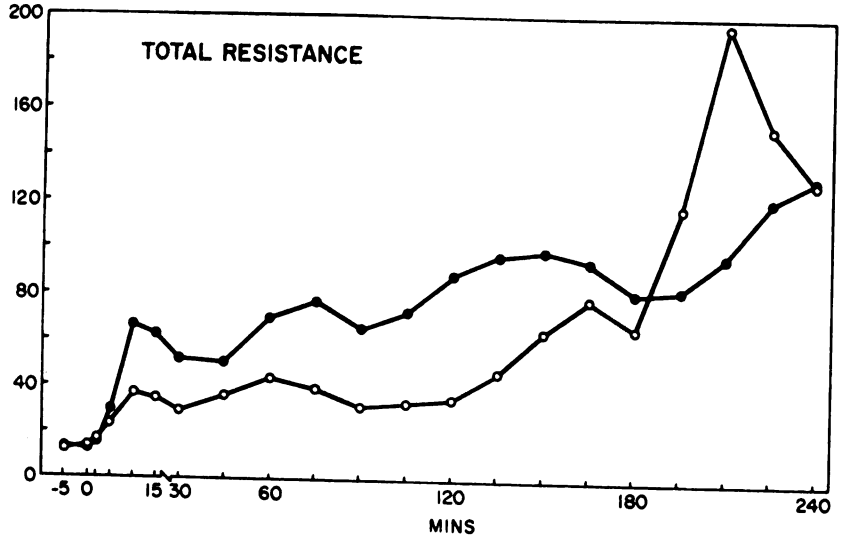
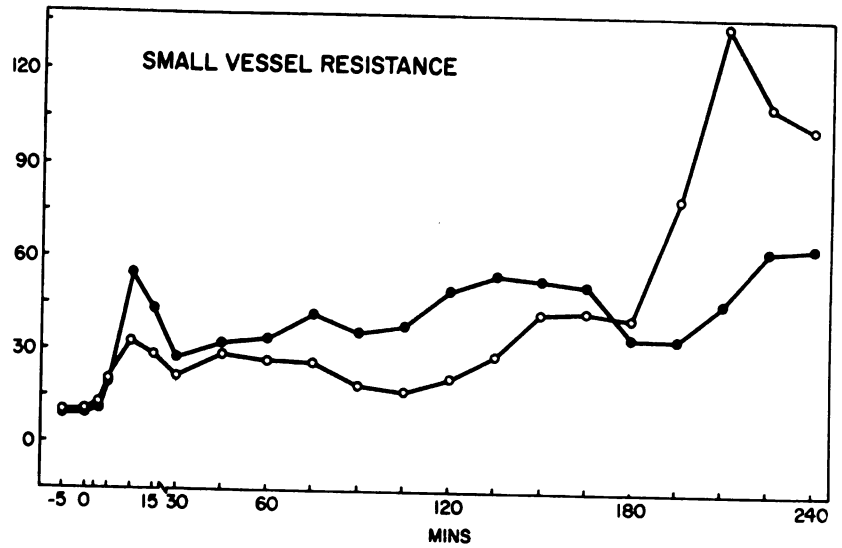
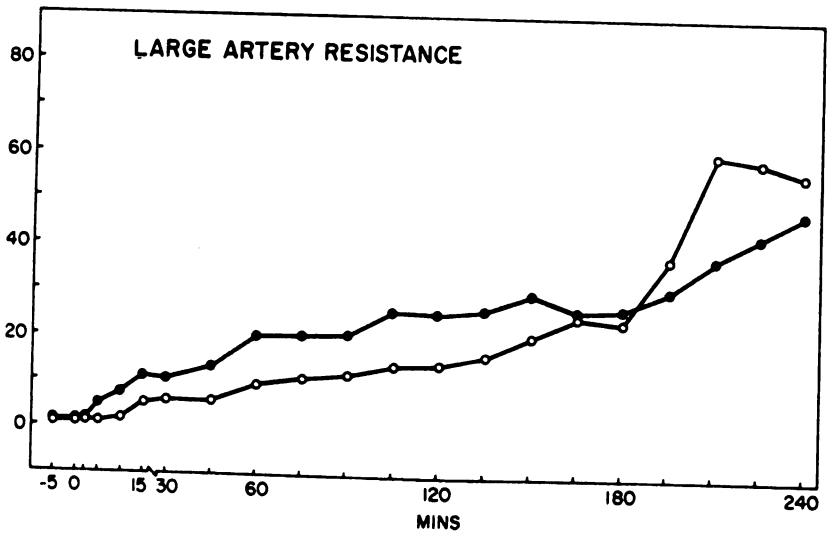

LARGE VEIN PRESSURE
8.0
6.0
4.0
2.0
0
-5 0
15 30
60
120
180
240
MINS

Figure 4. Effects of 2 mg/kg and 5 mg/kg endotoxin on total and segmental vascular resistances (mm Hg/ml/min/ 100g forelimb) in skin vasculature of dog forelimb. Symbols and N values correspond to those in Figure 1.

SKIN


TOTAL RESISTANCE
200
160
120
80
40
0
-5 0
15 30
60
120
180
240
MINS


SMALL VESSEL RESISTANCE
120
90
60
30
0
-5 0
15 30
60
120
180
240
MINS


LARGE ARTERY RESISTANCE
80
60
40
20
0
-5 0
15 30
60
120
180
240
MINS

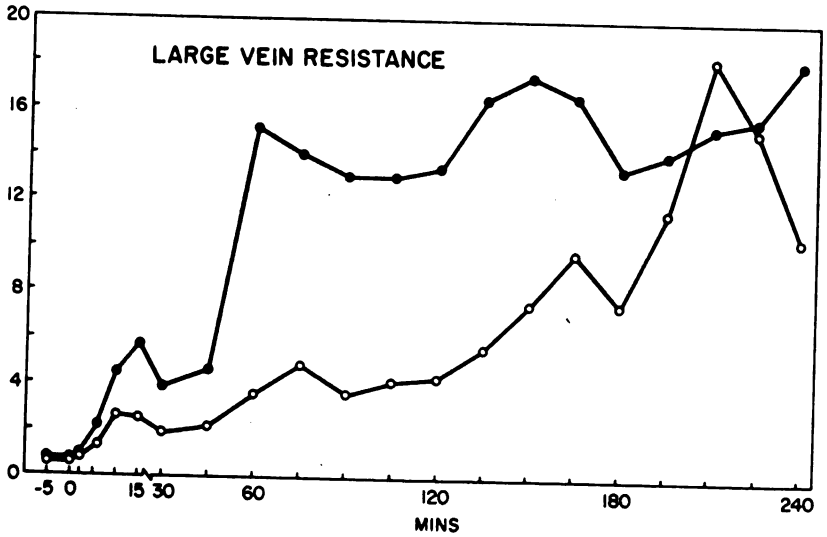
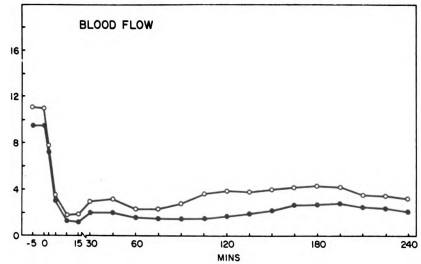
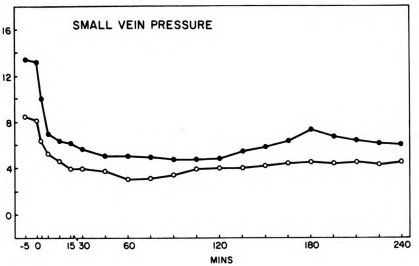
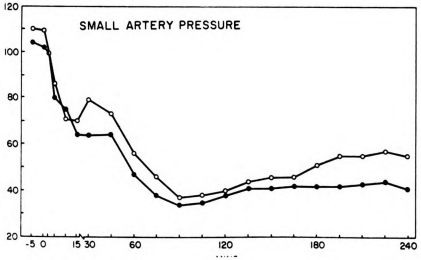

LARGE VEIN RESISTANCE
20
16
12
8
4
0
-5 0
15 30
60
120
180
240
MINS

Figure 5. Effects of 2 mg/kg and 5 mg/kg endotoxin on blood flow (ml/min/100g forelimb), and large and small vessel pressures (mm Hg) in muscle vasculature of the dog forelimb. Symbols and N values correspond to those in Figure 1.

MUSCLE


BLOOD FLOW
16
12
8
4
0
-5 0
15 30
60
120
180
240
MINS


SMALL VEIN PRESSURE
16
12
8
4
0
-5 0
15 30
60
120
180
240
MINS


SMALL ARTERY PRESSURE
120
100
80
60
40
20
-5 0
15 30
60
120
180
240

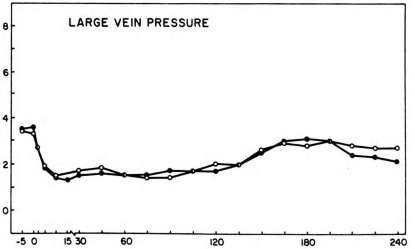
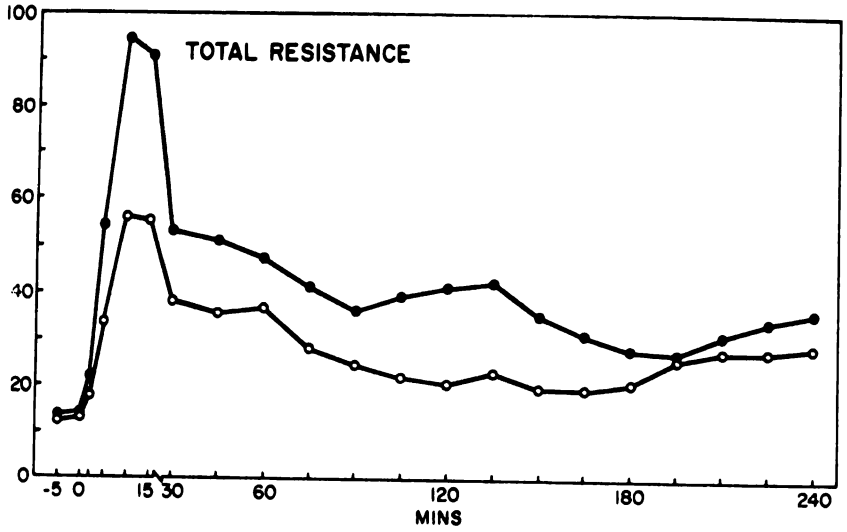
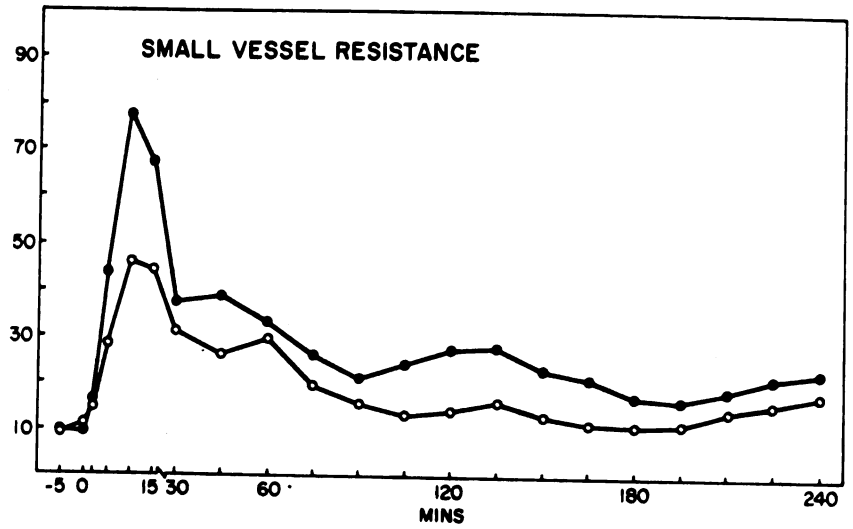
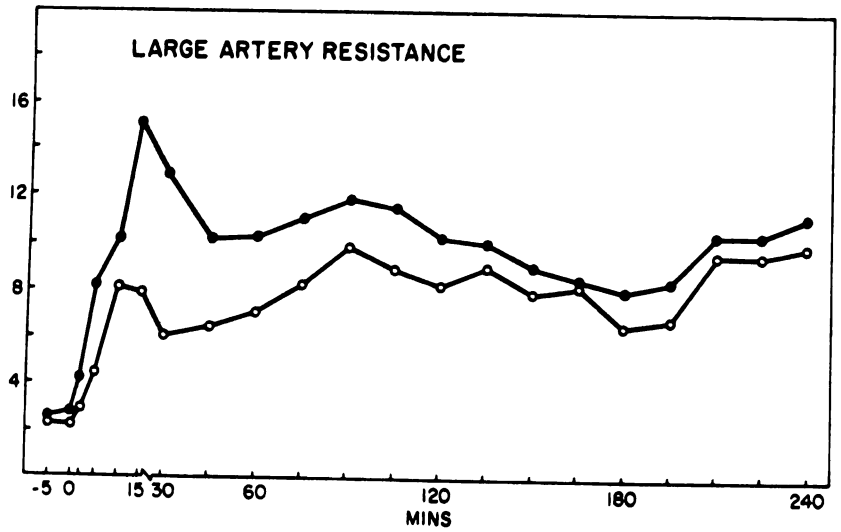

LARGE VEIN PRESSURE
8
6
4
2
0
-5 0
15 30
60
120
180
240

Figure 6. Effects of 2 mg/kg and 5 mg/kg endotoxin on total and segmental vascular resistances (mm Hg/ml/min/ 100g forelimb), in muscle vasculature of the dog forelimb. Symbols and N values correspond to those in Figure 1.

MUSCLE


TOTAL RESISTANCE
100
80
60
40
20
0
-5 0
15 30
60
120
180
240
MINS


SMALL VESSEL RESISTANCE
90
70
50
30
10
-5 0
15 30
60
120
180
240
MINS


LARGE ARTERY RESISTANCE
16
12
8
4
-5 0
15 30
60
120
180
240
MINS

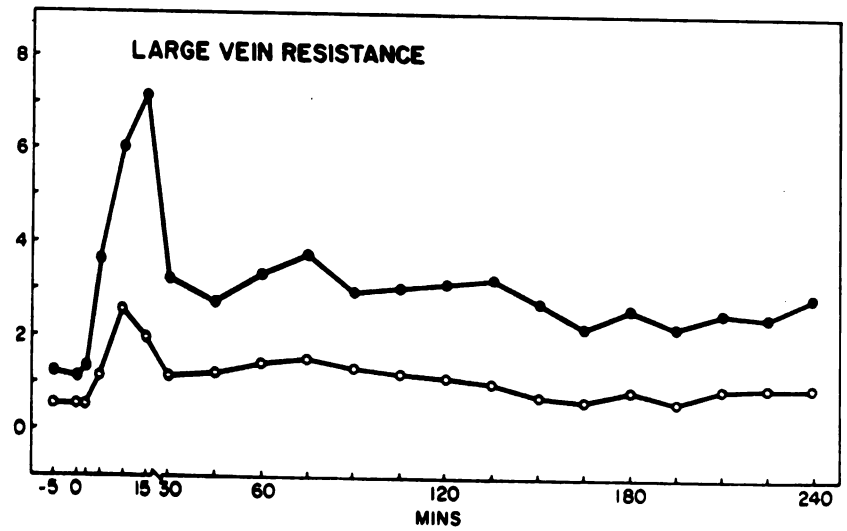

LARGE VEIN RESISTANCE
8
6
4
2
0
-5 0
15 30
60
120
180
240
MINS

TABLE 1. Effect of 2 mg/kg and 5 mg/kg endotoxin on electrolytes, osmolarity and hematocrit in systemic arterial blood.

		Experimental Period				
	Dose Level mg/kg	Control 0	60	120	180	240
Hematocrit	2	37.8	44.2*	45.2*	45.6*	46.1*
Hematocrit	5	38.5	45.8*	47.3*	46.8*	47.1*
Osmolarity	2	312	314	314	315*	316*
Osmolarity	5	309	313*	313*	313*	316*
Sodium	2	151	149	148*	151	151
Sodium	5	153	153	153	153	155
Potassium	2	3.4	3.6	3.7	3.9*	4.3*
Potassium	5	3.8	3.8	4.2	3.8	4.7*
Magnesium	2	2.1	2.0	2.1	2.1	2.2
Magnesium	5	1.8	1.7	1.8	1.8	1.9
Calcium	2	4.2	4.3	4.3	4.2	4.2
Calcium	5	4.4	4.6	4.5	4.6	4.2
pH	2	7.29	7.24*	7.23*	7.19*	7.19*
pH	5	7.29	7.26*	7.21*	7.22*	7.21*

* $p < 0.05$

TABLE 2. Mean change ± S.E. in forelimb weight, systemic pressure, skin and muscle vascular resistances in saline control animals (N=7)

	Control	Saline Infusion			
Minutes	0	60	120	180	240
		Δ Forelimb Weight (g)			
	0.0	-2.7 ±1.9	+0.4 ±0.7	+0.4 ±0.9	+0.7 ±1.0
		Systemic Pressure (mm Hg)			
	128 ±6.0	117 ±6.1	116 ±6.8	118 ±5.7	120 ±6.2
		Skin Vascular Resistances mm Hg/ml/min/100g			
Large Artery	2.3 ±0.6	3.1 ±0.8	4.0 ±1.1	3.9 ±1.3	4.3 ±1.0
Small Vessel	7.6 ±0.9	9.0 ±1.2	10.8 ±0.6	11.0 ±1.5	12.6 ±1.5
Large Vein	0.6 ±0.2	0.7 ±0.1	1.2 ±0.3	1.1 ±0.2	1.1 ±0.2
Total	11.0 ±1.2	13.1 ±1.2	15.8 ±1.8	16.0 ±1.7	18.0 ±2.4
		Muscle Vascular Resistances mm Hg/ml/min/100g			
Large Artery	3.0 ±0.8	4.1 ±1.1	3.9 ±0.9	4.1 ±0.7	5.0 ±1.3
Small Vessel	9.6 ±1.0	15.6 ±2.6	13.8 ±2.0	14.3 ±1.7	16.4 ±3.0
Large Vein	0.6 ±0.2	0.5 ±0.3	0.5 ±0.3	0.4 ±0.2	0.4 ±0.2
Total	13.0 ±1.9	20.0 ±1.6	17.7 ±2.7	18.5 ±2.8	23.6 ±3.2

TABLE 3. Mean change ± S.E. in systemic arterial pressure (SAP), gracilis vein pressure (Pv), blood flow (b.f.), and resistance (R) in gracilis muscle in response to 5 mg/kg/10 min endotoxin. (N=6. Mean muscle weight = 56.1 g.) and in saline controls (N=7).

MIN.	SAP (mm Hg)		Pv (mm Hg)		B.F. (ml/min)		R (mm Hg/ml/min/100g forelimb)	
	Endotoxin	Control	Endotoxin	Control	Endotoxin	Control	Endotoxin	Control
0	127.7 ±2.5	142.3 ±2.3	3.2 ±1.9	3.7 ±1.7	7.1 ±1.6	4.1 ±1.2	23.9 ±5.8	37.0 ±6.1
10	61.9 ±12.6	138.4 ±11.3	2.1 ±1.3*	3.6 ±1.5	4.4 ±1.5*	4.7 ±1.3	40.3 ±3.9*	31.9 ±4.2*
60	59.7 ±3.8*	141.7 ±6.1	1.9 ±0.9*	3.6 ±1.2	3.2 ±1.0*	4.4 ±1.4	24.2 ±10.1*	34.0 ±5.3*
120	69.6 ±7.5*	139.3 ±5.2	2.5 ±0.7*	3.6 ±1.0	4.0 ±1.2*	3.4 ±0.9*	25.2 ±7.1*	42.0 ±5.1*
180	69.8 ±4.4*	127.4 ±3.3*	1.9 ±0.1*	3.6 ±1.3	3.6 ±0.8*	2.8 ±1.1*	34.7 ±5.2*	47.3 ±4.8
240	66.5 ±5.4*	121.7 ±3.4*	1.5 ±0.3*	3.0 ±1.1*	3.3 ±0.8*	2.7 ±1.3*	26.1 ±6.2*	54.4 ±4.4*

* Sig. p = 0.05

CHAPTER V

DISCUSSION

Forelimb weight fell rapidly initially (0-10 min) and then more slowly throughout the remainder of a four hour period in response to 5 mg/kg endotoxin. The initial rapid weight loss also occurred in response to 2 mg/kg endotoxin, however weight remained relatively constant until min 180 and then decreased. Forelimb weight did not change significantly throughout a 4 hour period relative to control values in saline infused animals. Hinshaw and his co-workers (29) have also reported a continuous decrease in forelimb weight in autoperfused canine forelimbs in response to purified endotoxin (1 mg/kg) although the response was followed for only 120 minutes. The weight loss is theoretically attributable to a decreased vascular volume, interstitial fluid volume, intracellular fluid volume or some combination of the three. The weight loss, in the first 10 minutes, was associated with increases in all segmental vascular resistances. This suggests that mean vessel caliber was decreased, with a consequent decrease of vascular blood volume. From minutes 10-240 resistance in the capacitance vessel, i.e. small vessels and large veins, increased in skin but declined toward

control in muscle; the net effect being a fall in total forelimb resistance toward control. These responses suggest that mean vessel caliber was increasing during this time and that forelimb blood volume was also increasing. Hence, this weight loss could be due to a decrease in interstitial fluid volume and/or intracellular fluid volume.

The volume of the interstitial fluid compartment would decrease if the transmural hydrostatic pressure gradient fell or fell proportionately more than the transmural colloid osmotic pressure gradient. A decrease in the transmural hydrostatic pressure gradient is probable since all forelimb vascular pressures fell. Small vein pressure, which represents a minimum for Pc, was, in fact, significantly reduced throughout the experimental period. A fall in Pc would occur if the effect of a rise in postcapillary resistance was overwhelmed by the fall in aortic pressure and a rise in precapillary resistance.

It is possible that an intracellular fluid loss occurred, but it is also possible that intracellular fluid volume increased. Intracellular fluid volume is regulated by the extracellular-intracellular osmolarity gradient. Plasma osmolarity was observed to increase significantly in response to both dose levels of endotoxin in this study. If the extra osmotically active particles were added to the blood from organs or tissue other than the forelimb the increased gradient would promote intracellular dehydration. On the other hand, if they were added from the cells of the limb, the gradient

would be in the direction to promote intracellular hydration. Intracellular particle generation or failure of the sodium pump to promote sodium efflux from the cells subsequent to hypoxia has been suggested (42). Both would increase intracellular osmolarity and increase intracellular fluid volume. It is possible that the extra osmotically active particles were generated in the forelimb cells as well as in the cells of other organs, in which case a summing of the osmotic forces would determine fluid movement.

Although forelimb weight continuously decreased, it is nevertheless possible that microvascular permeability to plasma proteins increased. Fluid influx could still occur if the transmural hydrostatic pressure gradient fell to a lower level than the transmural colloid osmotic pressure gradient or, if the extracellular-intracellular osmolarity gradient changed in a direction which promoted fluid movement from tissue to blood. However, if microvascular permeability did increase, the increase was insufficient to cause net fluid efflux.

Constriction of all vascular segments contributed to the sustained increases in total skin and muscle vascular resistances (Figures 7 and 8). This was especially true in skin vasculature. In skin with both doses and in muscle with the high dose of endotoxin, the large arteries and large veins constricted almost proportionately. In muscle the low dose produced a proportionately greater rise in large artery resistance during the late part of the experimental period.

The combined large artery plus small vessel segment (prevenous) constricted proportionately more than the large vein segment during the later part of the period in response to the low dose in muscle. The large veins did not constrict proportionately more than the combined large artery plus small vessel segments in skin in response to either dose or in muscle in response to the high dose of endotoxin. In response to either dose of endotoxin, the percentage of total resistance in both the skin and muscle large artery segment increased (see Appendix Table 1). However, it is to be noted that the percentage of total resistance residing in the small vessel segment actually decreased from control (Figure 7, Appendix Table 1). The percentage of total resistance in the large vein segment increased in skin with both dose levels of endotoxin, but was unchanged relative to control in muscle. This indicates that constriction of the large artery segment in skin and skeletal muscle contributed greatly to the rise in precapillary resistance. Constriction of the large vein segment contributed greatly to the rise in postcapillary resistance in skin, but not in skeletal muscle.

The resistance increases during the first 10-15 minutes of endotoxin shock can be ascribed both to active changes, i.e. vascular smooth muscle contraction; passive changes, i.e. decreased transmural pressure; and possibly an increased blood viscosity (hematocrit). The continued increases in skin resistances during the remainder of the experimental period can be ascribed to active decreases in vessel caliber, since

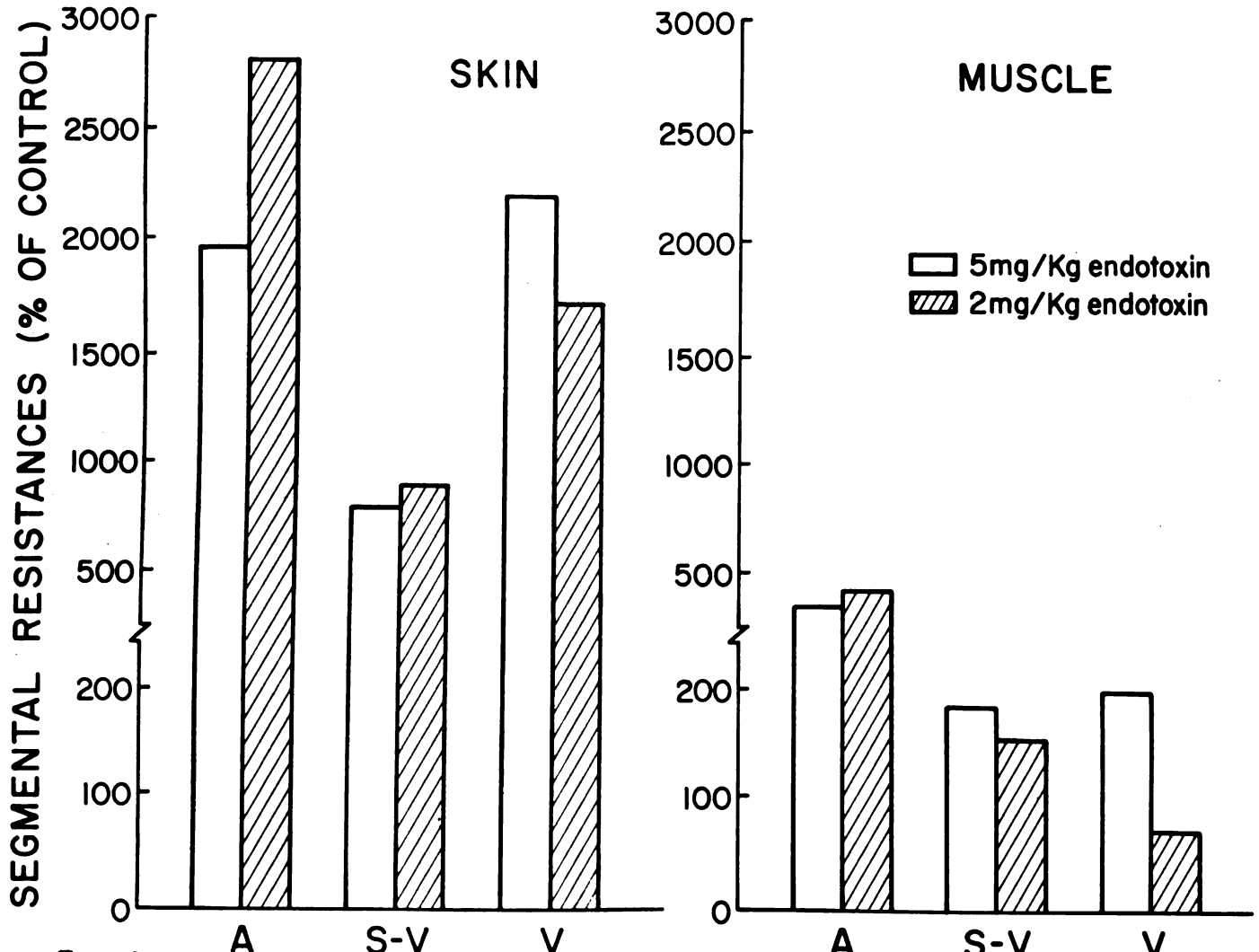


Figure 7. Segmental resistances in skin and muscle 4 hours after endotoxin administration expressed as percent change from control values.

average transmural pressures and hematocrit were nearly constant. However, muscle total, small vessel, and large vein resistances waned during the last three hours of the experimental period. This waning of muscle resistance was concurrent with a fall in transmural pressure, thus an active dilator must be involved. Since systemic pressure was constant or falling, the vasodilation was probably not due to a decreased baroreceptor stimulus. This decline in muscle resistance could have represented a failure of vasocontrictor nerve activity due to cerebral ischemia, a gradual failure of the local vasocontrictor mechanisms, or a greater accumulation of vasodilator metabolites, or a combination of the three. Various blood borne substances (4,5,39,40) have been suggested as contributing to this phenomenon, among them serotonin which increases skin resistances while having little effect on muscle resistances (3), and catecholamines, which then infused systemically at high dose levels, increase skin resistance proportionately more than skeletal muscle resistance (11). The observed increases in plasma $[K^+]$, $[H^+]$, and osmolarity support the concept that metabolic vasodilation contributed, at least partially, to the waning of muscle vascular resistances (14).

The response of the isolated gracilis muscle to endotoxin is essentially analogous to the response of skeletal muscle in the forelimb (Table 3). This suggests that the separation of skin and muscle blood flows with the forelimb technique is relatively complete. Total skin resistance in saline

animals only slightly increased (160%) during a 4 hour period, whereas an 1100% increase was seen in experimental animals at this time; hence, the spontaneous increase in skin resistance in the saline animals could account for a small fraction of the increase in skin resistance in the endotoxin animals (Figure 8 and Table 2). Total muscle resistance increased 180% after 4 hours in saline treated animals and 260% in experimental animals at this time. In gracilis experiments total muscle resistance increased 145% after 4 hours in saline controls and 270% in animals given endotoxin at this same time (Table 3). Hence, in muscle this spontaneous resistance increase could represent a significant part of the total resistance increase in endotoxin animals.

These observations suggest that endotoxin in shock is associated with marked skin vascular constriction, but only small transient muscle vascular constriction. Data from the gracilis muscle of animals infused with saline or endotoxin also suggests that endotoxin shock is associated with transient, weak vascular constriction in skeletal muscle.

These data demonstrate that in the forelimb during endotoxin shock an important compensatory mechanism, that of extravascular fluid reabsorption, is well maintained. The findings of this study fail to support the contention that fluid loss into forelimb skin and skeletal muscle is an important determinant of irreversibility in endotoxin shock states.

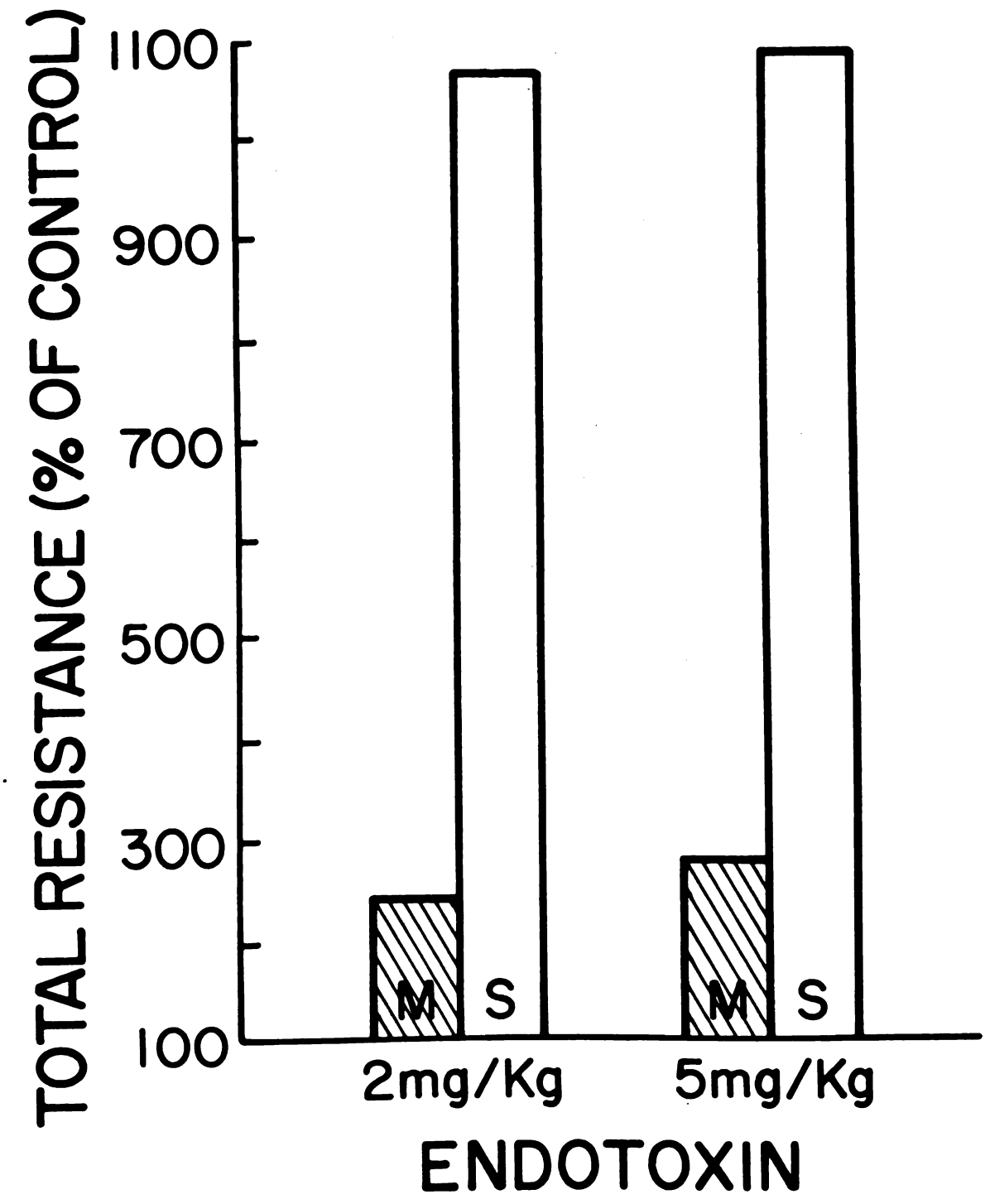


Figure 8. Total skin and muscle resistances 240 minutes after endotoxin administration expressed as percent change from control values.

CHAPTER VI

SUMMARY

E. coli endotoxin (2 mg/kg or 5 mg/kg) administered i.v. to dogs produced sustained decreases in forelimb skin and skeletal muscle vascular pressures and blood flows; segmental vascular resistance (large artery, small vessel, large vein) increased, especially in skin. Forelimb weight decreased throughout a 4 hour period. An initial period (0-10 min) of rapid weight loss is largely attributable to a decreased vascular volume subsequent to constriction of forelimb capacitance vessels. The slow weight loss (10-240 min) is associated with further resistance increases in skin capacitance vessels and decreases toward control in skeletal muscle capacitance vessels; the net effect being a fall in total forelimb vascular resistance toward control. These data suggest an increasing forelimb vascular volume from minutes 10-240 and, that the weight loss over this period is attributable to extravascular fluid reabsorption from the interstitial and/or intracellular compartments. The fluid influx may have occurred subsequent to a fall in Pc, since small vein pressures, which represent a minimum for Pc, were decreased in skin and skeletal muscle from minutes 10-240.

The osmolarity of arterial plasma increased; this could promote cellular hydration or dehydration depending on the origin of the extra osmotically active particles.

The results of this study do not support the contention that net fluid efflux into skin and skeletal muscle is a determinant of irreversibility in endotoxin shock. On the contrary, this study demonstrates that during canine endotoxin shock, the reabsorption of extravascular fluid continues.

BIBLIOGRAPHY

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APPENDIX

TABLE A1. Percent of total resistance residing in vascular segments at control and at min 240 in response to 2 mg/kg and 5 mg/kg endotoxin.

	LARGE ARTERY		SMALL VESSEL		LARGE VEIN	
	CONTROL	MIN 240	CONTROL	Min 240	CONTROL	MIN 240
ENDOTOXIN (2 mg/kg)						
SKIN	14%	29%	81%	57%	5%	14%
MUSCLE	17%	36%	78%	60%	5%	4%
ENDOTOXIN (5 mg/kg)						
SKIN	19%	38%	74%	48%	7%	14%
MUSCLE	19%	31%	74%	62%	7%	7%

Key to Tables A2 and A3

Complete date from forelimb endotoxin experiments (2 mg/kg and 5 mg/kg).

All data given as mean value ± standard error (at minutes 0, 10, and 240). The following abbreviations were used:

Δwtg = change in forelimb weight

A.P. = systemic arterial pressure

Pssv = small skin vein pressure

Plsv = large skin vein pressure

Pssa = skin artery pressure

Psmv = small muscle vein pressure

Plmv = large muscle vein pressure

Psma = muscle artery pressure

Fs/100g = skin blood flow per 100g forelimb

Fm/100g = muscle blood flow per 100g forelimb

Rsa = skin artery resistance

R(s-v)s = skin small vessel resistance

Rsv = skin vein resistance

Rst = total skin resistance

Rms = muscle artery resistance

R(s-v)m = muscle small vessel resistance

Rmv = muscle vein resistance

Rmt = total muscle resistance

Rat = total arterial resistance

R(s-v)t = total small vessel resistance

Rvt = total venous resistance

Rt = total resistance

S(la/lv)r = skin (large artery/large vein) resistance ratio

M(la/lv)r = muscle (large artery/large vein) resistance ratio

S(Preven/ven)r = skin (prevenous/venous) resistance ratio

M(Preven/ven)r = muscle (Prevenous/venous) resistance ratio

Tpsa = skin arterial transmural pressure

Tp(s-v)s = skin small vessel transmural pressure

Tpsv = skin venous transmural pressure

Tpma = muscle arterial transmural pressure

Tp(s-v)m = muscle small vessel transmural pressure

Tpmv = muscle venous transmural pressure

Δwt is given in grams (g); all pressures in mm Hg; blood dlows in ml/min/100g forelimb; resistance in mm Hg/ml/min/ 100g forelimb.

TABLE A2. Endotoxin 2 mg/kg/10 min infusion. N=10 Weight 413.0 ± 19.7g.

	▵wt	A.P.	Pssv	Plsv	Pssa	Fs/100g	Psmv	Plmv	Psma	Fm/100g	Rsa	Rs(s-v)
-5	0.0	130	10.5	4.3	109	15.2	8.5	3.4	110	11.1	1.8	10.4
0	0.2	130	10.2	4.1	108	15.2	8.2	3.3	109	11.0	1.9	10.9
	±0.1	±5.0	±1.5	±1.2	±6.8	±3.1	±1.0	±0.7	±5.0	±1.0	±0.3	±2.7
2	-1.7	117	7.9	2.9	98	12.8	6.4	2.7	99	7.8	2.1	14.3
5	-4.3	99	6.6	0.8	86	7.6	5.3	1.9	86	3.5	2.2	2.2
10	-6.5	84	4.5	-0.5	72	5.0	4.6	1.5	71	1.8	3.9	32.0
	±0.8	±7.7	±1.0	±0.8	±9.0	±1.3	±0.7	±0.9	±10.0	±0.3	±1.0	±8.0
15	-7.8	82	4.4	-0.2	68	5.2	4.0	1.7	70	1.9	5.1	29.1
30	-8.5	93	5.3	0.5	75	6.3	4.0	1.8	79	3.0	5.9	23.4
45	-8.5	89	5.0	-0.0	72	4.7	3.8	1.5	73	3.2	5.7	29.0
60	-9.2	69	5.0	-0.6	55	3.6	3.1	1.4	56	2.3	9.1	27.3
75	-9.4	57	4.4	-0.2	40	2.9	3.2	1.4	41	2.3	10.5	26.2
90	-9.3	54	4.6	-0.2	35	2.7	3.5	1.7	37	2.8	11.4	17.8
105	-9.0	58	5.3	0.2	36	3.2	4.0	1.9	38	3.6	13.4	16.1
120	-9.2	58	5.8	0.5	37	2.9	4.1	2.0	40	3.9	13.6	19.0
135	-9.2	63	5.8	0.3	40	2.9	4.1	2.0	44	3.8	15.4	29.5
150	-9.2	67	5.4	0.0	42	3.0	4.3	2.6	46	4.0	20.0	43.0
165	-9.2	70	5.8	0.1	43	3.0	4.5	2.9	46	4.2	24.2	43.3
180	-9.3	72	5.7	-0.2	45	3.1	4.6	2.8	51	4.3	23.0	41.8
195	-9.7	77	5.8	-0.4	49	2.6	4.5	3.0	55	4.2	37.0	81.6
210	-10.5	78	5.0	-0.6	48	2.4	4.6	2.8	55	3.5	59.3	142.4
225	-11.3	79	4.8	-0.7	51	2.1	4.4	2.7	57	3.4	48.5	109.0
240	-12.5	78	4.9	-0.6	50	2.0	4.6	2.7	55	3.2	45.4	88.4
	±3.5	±8.4	±1.0	±1.0	±9.2	±0.4	±0.9	±0.8	±11.0	±0.7	±12.1	±26.6

TABLE A2 (Continued)

	Rsv	Rst	Rms	Rm(s-v)	Rmv	Rmt	Ft/100g	Rat	R(s-v)t	Rvt	Rt	Sra/rv
-5	0.6	12.2	2.2	9.7	0.5	12.5	26.3	0.9	4.9	0.25	5.7	4.8
0	0.6 ±0.2	12.8 ±3.0	2.2 ±0.2	10.2 ±1.6	0.5 ±0.1	12.8 ±1.3	26.0 ±2.7	1.0 ±0.1	5.2 ±0.9	0.25 ±0.1	5.9 ±0.9	4.9 ±1.0
2	0.8	16.2	2.8	14.5	0.5	17.5	20.8	1.1	7.1	0.29	7.6	5.4
5	1.3	23.1	4.4	27.7	1.1	33.7	11.1	1.3	11.8	0.6	12.6	3.5
10	2.6 ±0.9	36.6 ±8.2	8.0 ±1.0	46.1 ±7.6	2.5 ±0.1	55.2 ±7.0	6.7 ±1.5	2.3 ±0.5	18.4 ±2.9	1.3 ±0.3	19.4 ±4.1	3.3
15	2.5	34.3	7.8	44.0	1.9	50.0	7.1	2.5	16.4	0.9	17.4	4.0
30	1.9	29.7	6.0	30.4	1.1	37.7	9.3	2.4	12.8	0.5	14.6	5.2
45	2.2	35.6	6.4	26.7	1.2	35.1	7.9	2.6	13.9	0.7	15.2	5.5
60	3.6	43.4	7.0	29.7	1.4	36.5	5.8	3.0	14.2	0.9	15.2	5.2 ±1.9
75	4.9	39.1	8.2	19.6	1.5	27.8	5.3	3.6	10.2	1.0	13.6	7.6
90	3.7	31.2	9.8	15.4	1.3	24.6	5.5	4.1	7.5	0.8	11.5	9.4
105	4.2	31.8	8.9	13.7	1.2	21.9	6.8	4.0	6.8	0.8	10.7	7.3
120	4.4	34.7	8.2	14.5	1.1	21.4	6.9	3.6	7.3	0.7	10.7	7.0 ±1.9
135	5.7	47.1	9.0	15.7	1.0	23.3	6.7	3.6	7.7	0.7	11.0	7.2
150	7.6	64.4	7.9	12.9	0.7	19.9	7.1	3.4	7.2	0.5	10.1	7.4
165	8.9	78.9	8.2	11.7	0.6	19.5	7.2	3.9	6.7	0.4	10.0	7.9
180	7.6	67.0	6.5	11.5	0.8	20.7	7.4	3.4	6.6	0.5	10.0	6.8 ±1.8
195	12.7	119.0	6.8	11.7	0.6	25.3	6.8	3.7	7.8	0.5	11.5	6.2
210	18.3	197.7	9.5	14.4	0.8	27.1	6.0	4.3	8.5	0.5	12.8	8.4
225	13.2	153.0	9.5	15.8	0.8	27.1	5.5	4.7	9.3	0.6	13.8	7.9
240	10.5 ±3.3	130.4 ±30.0	9.9 ±4.4	17.0 ±4.4	0.8 ±0.3	28.3 ±4.4	5.2 ±0.6	5.1 ±0.8	9.0 ±1.6	0.6 ±0.2	14.0 ±1.3	7.1 ±1.9

TABLE A2 (Continued)

	Mra/rv	SPreven/ven	MPreven/ven	Tpsa	Tp(s-v)s	Tpsv	Tpma	Tp(s-v)	Tpmv
-5	4.2	24	25	121	59	7.4	121	60	5.6
0	4.7 ±0.6	24 ±3.2	27 ±4.6	121	59	7.2	121	60	5.5
2	4.6	26	30	108	53	5.4	108	54	4.3
5	3.8	21	30	93	46	3.7	93	50	3.3
10	3.3	22	26	78	38	2.0	77	39	2.8
15	3.9	24	30	75	36	2.2	76	38	2.5
30	5.2	28	32	85	40	2.9	87	43	2.6
45	5.5	25	32	82	39	2.5	82	40	2.3
60	5.2 ±0.8	23 ±6.4	32 ±4.8	63	30	2.2	63	40	1.9
75	5.5	22	24	48	22	2.1	49	30	1.9
90	7.5	22	23	44	20	2.2	45	23	2.4
105	7.6	16	24	46	20	2.7	47	21	2.8
120	7.2 ±1.4	16 ±4.7	24 ±3.3	46	21	3.1	48	22	2.8
135	8.2	16	30	51	23	3.1	53	24	2.9
150	8.8	17	32	54	24	2.7	55	26	3.3
165	10.0	17	37	55	24	2.9	58	28	3.5
180	9.8 ±1.2	16 ±4.7	38 ±5.3	57	25	2.7	60	30	3.5
195	11.5	15	44	61	27	2.7	65	32	3.5
210	9.6	18	45	62	26	2.2	65	32	3.5
225	11.3	18	45	64	27	2.0	67	33	3.4
240	11.0 ±1.4	17 ±3.9	45 ±7.2	63	27	2.1	66	32	3.5

TABLE A3. Endotoxin 5 mg/kg/10 min infusion. N=10 Weight 444 ± 28.7g.

	▵wtg	A.P.	Pssv	Plsv	Pssa	Fs/100g	Psmv	Plmv	Psma	Fm/100g	Rsa	R(s-v)s
-5	0	128	14.4	7.0	99	12.0	13.4	3.5	104	9.5	2.4	9.1
0	0.1 ±0.1	131 ±5.2	14.5 ±2.0	7.0 ±2.3	99 ±7.2	12.1 ±1.8	13.2 ±1.7	3.6 ±0.6	102 ±4.0	9.5 ±0.5	2.5 ±0.3	9.1 ±1.8
2	-1.7	121	11.7	4.5	96	9.6	10.0	2.7	99	7.2	2.8	10.7
5	-5.1	95	8.5	1.4	78	4.5	7.0	1.8	80	3.0	5.0	21.3
10	-8.5 ±2.7	84 ±7.1	5.7 ±1.0	0.5 ±0.3	72 ±6.0	2.5 ±0.8	6.4 ±1.0	1.4 ±0.6	75 ±6.1	1.3 ±0.3	7.5 ±2.3	54.0 ±13.0
15	-10.4	76	5.6	0.2	58	2.7	6.2	1.3	64	1.2	12.1	44.3
30	-10.7	82	6.2	0.7	60	3.6	5.7	1.5	64	2.0	10.4	27.0
45	-11.5	79	5.7	-0.2	56	2.9	5.1	1.6	64	2.0	13.3	32.4
60	-13.1	60	6.1	-0.2	43	2.0	5.1	1.5	47	1.6	20.0	33.7
75	-14.3	50	5.8	-0.1	34	1.8	5.0	1.5	38	1.5	20.0	42.8
90	-15.8	48	5.3	-0.2	31	1.6	4.8	1.7	34	1.5	20.7	34.6
105	-17.0	49	5.2	-0.1	30	1.4	4.8	1.7	35	1.5	25.1	37.6
120	-18.0	50	6.3	0.3	33	1.3	4.9	1.7	38	1.6	25.0	51.3
135	-18.4	54	7.1	1.0	36	1.3	5.5	2.0	41	1.8	25.7	55.8
150	-18.3	56	7.7	1.3	37	1.3	5.9	2.5	41	2.2	28.6	53.7
165	-18.3	57	7.8	1.8	36	1.5	6.4	3.0	42	2.6	25.8	52.6
180	-18.3	59	8.3	1.7	35	1.5	7.4	3.1	42	2.7	26.2	33.3
195	-18.8	60	8.4	1.8	35	1.4	6.8	3.0	42	2.8	30.2	32.8
210	-19.9	61	7.8	1.3	37	1.2	6.5	2.4	43	2.5	37.1	45.7
225	-20.9	63	7.8	1.2	38	1.0	6.2	2.3	44	2.3	42.7	63.6
240	-21.7 ±3.8	59 ±7.0	7.4 ±1.4	0.6 ±1.8	33 ±6.0	0.9 ±0.3	6.1 ±1.0	2.1 ±0.8	41 ±6.8	2.1 ±0.4	47.5 ±10.4	63.8 ±19.2

TABLE A3. (Continued)

	Rsv	Rst	Rms	R(s-v)m	Rmv	Rmt	Ft/100g	Rat	R(s-v)t	Rvt	Rt	S(la/lv)r
-5	0.8	12.4	2.5	9.9	1.2	13.7	21.4	1.2	4.2	0.4	6.0	5.1
0	0.8 ±0.2	12.5 ±1.8	2.7 ±0.2	9.6 ±0.7	1.1 ±0.2	13.8 ±1.0	21.7 ±1.9	1.3 ±0.1	4.2 ±0.6	0.4 ±0.1	6.1 ±0.5	5.5 ±1.2
2	1.0	14.5	4.2	16.2	1.3	21.8	16.8	1.6	5.5	0.4	7.7	4.3
5	2.1	28.4	8.1	43.3	3.6	54.9	7.5	2.9	13.3	0.9	17.4	3.3
10	4.5 ±1.1	65.8 ±17.4	10.4 ±4.0	77.7 ±17.8	6.0 ±1.2	94.1 ±21.2	3.9 ±0.9	4.2 ±1.2	29.0 ±8.1	2.1 ±0.5	36.5 ±9.3	3.0
15	5.6	62.0	15.6	67.6	7.1	90.3	3.9	6.2	23.2	2.1	33.0	3.8
30	3.9	51.3	12.8	37.4	3.2	53.5	5.6	5.6	14.8	1.2	22.1	6.3
45	4.6	50.3	10.1	38.8	2.7	51.6	4.9	5.7	15.8	1.1	24.0	6.6
60	15.1	68.6	10.2	33.8	3.3	47.3	3.5	5.9	17.2	1.5	25.6	5.3
75	14.0	77.1	11.0	26.2	3.7	41.0	3.2	6.7	12.7	1.6	21.8	+2.0 −5.3
90	13.1	65.5	11.8	21.3	2.9	36.1	3.1	7.1	11.1	1.4	20.3	4.8
105	13.1	72.4	11.5	24.7	3.0	39.1	2.9	7.2	12.5	1.4	22.2	5.5
120	13.5	89.7	10.2	27.6	3.1	40.9	2.9	6.2	14.4	1.5	23.4	5.3
135	16.5	97.9	10.0	27.7	3.2	42.0	3.0	6.4	15.2	1.5	24.2	+1.9 −5.4
150	17.5	99.8	9.0	23.4	2.7	35.1	3.5	5.8	12.9	1.3	20.9	7.2
165	16.6	95.0	8.5	21.4	2.2	31.0	4.1	5.0	12.2	1.1	19.0	8.6
180	13.5	82.0	8.0	17.2	2.6	27.9	4.3	5.5	8.5	1.3	16.1	+3.9 −8.7
195	14.2	84.2	8.4	16.8	2.2	27.5	4.2	5.8	8.7	1.2	16.3	9.2
210	15.3	98.1	10.4	18.2	2.5	31.1	3.6	6.7	9.9	1.3	19.0	9.1
225	15.6	122.0	10.4	21.2	2.4	34.0	3.2	7.2	12.3	1.3	21.6	9.6
240	18.2 ±7.0	131.6	11.2 ±1.2	21.6 ±3.7	2.8 ±0.6	35.7 ±4.2	3.0 ±0.6	8.0 ±0.8	12.5 ±2.7	1.6 ±0.3	23.2 ±3.0	6.2 ±2.0

TABLE A3. (Continued)

	M(la/lv)r	S(Preven/ven)r	M(Preven/ven)r	Tpas	Tp(s-v)s	Tpsv	Tpma	Tp (s-v)m	Tpmv
-5	3.3	19.6	15.1	113	56	11	116	59	8
0	4.0 ±1.0	19.9 ±3.3	16.2 ±3.4	115	57	11	117	58	8
2	3.6	18.4	17.6	109	54	8	110	55	6
5	3.7	16.2	18.6	87	43	5	88	44	4
10	1.9	17.7	17.2	78	38	3	80	41	4
15	3.0	15.1	21.3	67	32	3	70	35	4
30	4.8	21.4	22.5	71	33	3	73	35	4
45	4.6	17.6	24.0	68	31	3	71	35	3
60	4.4 ±1.2	12.7 ±3.2	22.4	52	25	3	54	26	3
75	3.8	11.4	15.6	43	20	3	44	22	3
90	5.2	10.2	17.0	39	18	3	41	19	3
105	5.0	11.1	16.0	39	17	3	42	20	3
120	4.0 ±0.7	11.3 ±4.0	15.7	42	19	3	44	22	3
135	4.1	11.8	15.9	45	21	4	48	23	4
150	4.7	13.2	16.9	46	22	5	49	24	4
165	6.1	16.3 ±4.3	21.2	47	22	5	50	24	5
180	4.9 ±1.6	14.5	16.0	47	22	5	50	25	5
195	5.3	16.6	16.4	48	22	5	51	25	5
210	4.9	15.7	15.4	49	22	5	53	25	4
225	5.7	15.6	19.4	51	22	4	54	25	4
240	5.2 ±1.0	15.6 ±4.4	15.5	47	20	4	50	23	4