HEMODYNAMICS OF THE RAT AND MOUSE

by

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ABSTRACT

There are two theoretical concepts of vertebrate hemodynamics, expressed by the Windkessel and Tubular models. The "Windkessel" is a lumped parameter system in which pressure pulse wave velocity is infinite and wave reflection is impossible. Conceptually, the central arterial vessels cushion flow pulsations while the peripheral arterioles act simply as conduits. In this system, diastolic pressure falls exponentially and wave shapes and amplitudes are identical throughout the arterial tree. In the "Tubular" model, wave velocity is finite and reflected waves augment peripheral pressure and reduce peripheral flow pulsations. Diastolic pressure decline is interrupted by secondary fluctuations and wave shapes and amplitudes are altered during travel. Peripheral resistance (Rs) effects the magnitude of wave reflections while pressure pulse wave velocity (C), cardiac frequency (f₀), and arterial length (L), interact to define their effect on the heart. Simply, f₀ produces oscillating waves where incident and reflected wave summation create nodal and antinodal points in the central aorta. The correct matching of L and C/f₀ places the heart near a pressure node, thereby uncoupling it from the high terminal impedance. However, incorrect matching may promote an early, detrimental return of reflections to the heart. In most mammals, appropriate L/C/f₀ matching reduces systemic impedance, however, this may not occur in very small animals. Simultaneous measurements of aortic arch pressure and flow and abdominal aortic pressures were made under varying physiological conditions
in rats and mice. These measurements were used to identify waveform alterations and to calculate systemic impedance in each species. Distal aortic wall properties were described by biomechanical tests. There were no significant differences in arterial mechanicals or anatomy in the rat as compared with other mammals. Cardiovascular indices measured in anesthetized open-chest, ventilated rats were similar to both allometrically estimated, and previously reported, values. In contrast, the abdominal aorta of the mouse was more distensible, measured mean arterial pressure lower, and C slower, than in the rat. Stroke volume was greater, \( f_0 \) slower, and Rs less than were estimated allometrically. While wave reflections were observed in the mouse their effects were not discrete. Despite a mismatch in \( L/C/f_0 \) inappropriate return of reflections to the heart was prevented by the decrease in Rs and aortic stiffness. While the hemodynamics of the rat can be described by the Tubular model, the systemic impedance of the mouse is minimized primarily through a combination of low Rs and increased central arterial distensibility.
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2. \( D = 1 / E_{\text{eff}} (h/d) \) where \( E_{\text{eff}} \) is Young’s effective incremental modulus (kg s\(^{-2}\) m\(^{-1}\)), \( h \) is arterial wall thickness (mm), and \( d \) is external diameter (mm)

3. \( C_o (m/s)= (E_{\text{eff}} h / \rho d)^{1/2} \) where \( E_{\text{eff}} \) is Young’s effective incremental modulus (kg s\(^{-2}\) m\(^{-1}\)), \( h \) is arterial wall thickness (mm), \( \rho \) is blood density (kg m\(^{-3}\)) and \( d \) is outside vessel diameter (mm)

4a) Damped frequency \( (f_d) = 1 / T_d \) where \( T_d \) is the elapsed time from the first to the second oscillatory trough and,

4b) Damping Coefficient \( (\delta) = \Lambda / (4\pi^2 + \Lambda^2)^{0.5} \)

where \( \Lambda \) is equal to \( \log_e (d_1/d_2) \), and \( d_1, d_2 \) are the first and second oscillatory peaks, respectively.

5. Mean Arterial Pressure (MAP, mmHg) = \( [(\text{Systolic} - \text{Diastolic}) / 3] + \text{Diastolic} \)

6. Pulsatile Pressure (PP, mmHg) = Peak Systolic Pressure - Minimum Diastolic Pressure

7. Velocity \( (V, \text{mm/s}) = (F_d * C) / (2 * F_o * \cos A) \) where \( F_d = \) doppler shift in kHz, \( C = \) velocity of sound in blood in mm/s, \( F_o = \) transmitter frequency in kHz, \( A = \) angle between the probe and the \( V \) vector.

8. Cardiac Output, CO (cm\(^3\)/min) = \( (V \text{ cm/s} * \pi D \text{ cm/2})^2 (60 \text{s/min}) \)

9. Stroke Volume, SV (cm\(^3\)/b) = CO (cm\(^3\)/min) / Heart Rate (b/m)

10. Peripheral Resistance PRU (dynes s\(^{-1}\) cm\(^3\)) = \( [(\text{MAP dynes} / 10) (980 \text{ cm} / \text{s}^2)] / (\text{CO cm}^3 / \text{s}) \)

11. Pulse Wave Velocity (C, in m/s) = \( \Delta Z / \Delta t \)

12. Pressure Pulse Wavelength \( \lambda \) (in frequency domain)
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14a) Impedance \( (Z_n, \text{dyn s/cm}^2) = \text{Pressure} (P_n, \text{dyn s/cm}^3) / \text{Flow} (Q_n, \text{cm/s}), \) where \( n = \) harmonic frequency

14b) Phase \( (\theta_n, \text{radians}) = \text{Flow phase} (\beta_n, \text{radians}) - \text{Pressure phase} (\alpha_n, \text{radians}) \)
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15. Theoretical Input Impedance predicted according to the 3-Component Windkessel Model
\[ Z_{in} = R_c + \left( R_a / \left\{ 1 + (j\omega C_a R_a) \right\} \right) \]

where \( Z_{in} \) = impedance modulus at each \( \omega \) (\( \omega = 2\pi f \), \( f \) is heart rate in b/s), \( R_c \) = characteristic impedance determined from arithmetic mean of \( Z \) amplitudes from the 1st to nth harmonic, \( R_a \) = difference between 0 harmonic impedance and \( R_c \), \( j = \sqrt{-1} \), and \( C_a = T / R_a \), where \( T \) is equal to the diastolic time decay of the pressure pulse.

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where \( \bar{R} = \{ \text{Outside vessel radius (R, mm) + Inside vessel radius (r, mm)} \} / 2 \)

19. Incremental Elastic Modulus \( (E_{inc}, \text{ in kPa}) = (1 - \mu^2)(1 + \epsilon)(\Delta \sigma_c / \Delta \epsilon) \), where \( \mu \) = Poisson's ratio, assumed to be 0.5 for arterial vessel walls (Bergel, 1963), \( \Delta \sigma \) is the change in circumferential wall stress and \( \Delta \epsilon \) is the change in circumferential wall strain at each pressure increment.

20. Lamellae-Thickness Ratio
\( L_m : h = \text{(Number of lamellae)} / \text{(wall thickness, mm)} \)

21. Predicted Pulse Wave Velocity \( (C_o, \text{ in m/s}) = \left[ (E_{inc}, \text{ dyn-s/cm}^2)(h, \text{cm}) / (2\alpha, \text{ dyn s/cm}^2)(R_{in}, \text{ cm}) \right] / 100 \)

22. Predicted Transit Time as Percentage of Cardiac Cycle
\( \%CC_{pred} = \left[ (L, \text{ m} / C_o, \text{ m/s}) / (1 \text{ sec} / f_o, \text{ b/s}) \right] * 100 \)

ALLOMETRIC FORMULATIONS

23. Heart Rate (b/s) = 3.93 \( M^{0.25} \)

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26. Aortic Length (m) = \( M^{-0.33} \)
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GENERAL INTRODUCTION

The design of the systemic arterial tree and its influence on the relationship between blood pressure and blood flow has intrigued investigators since the circulation of the blood was first described by William Harvey (1578-1657). The physical design of the heart dictates that blood pressure hence, blood flow, is pulsatile in nature yet the conversion of cardiac output into a smooth peripheral flow is a fundamental aspect of all mammalian circulations. The reduction of flow pulsatility and maintenance of adequate perfusion pressure is accomplished through the intricately correlated functions of the heart and systemic vasculature (Hamilton, 1963; Haynes, 1963; Taylor, 1973).

The examination of pressure and flow waveform recordings obtained from numerous mammalian species have provided a fundamental knowledge of the characteristics of arterial wave travel which are central to circulatory function. While ascending aortic flow waves are nearly identical in all species studied, pressure waveforms are noticeably different (Attinger et al., 1966; Avolio et al., 1985; Li and Noordergraaf, 1991). When blood is ejected from the left ventricle into the compliant ascending aorta during systole, the vessel wall expands to accommodate the increase in blood volume. As cardiac ejection slows and pressure begins to fall, the distended wall returns to its equilibrium position and the recoil energy exerted against the blood drives it forward expanding the next portion of the vessel. In this manner, a pressure pulse wave (incident wave) is propagated away from the
heart. As the wave travels toward the peripheral vessels, alterations in vessel properties and branching points cause portions of it to be reflected backwards (reflected waves). The interactions of these incident and reflected wave elements shape the pressure pulse profile and alter the pulsatile pressure amplitude at all points within the arterial tree. (Aperia, 1940; McDonald and Attinger, 1964; Guyton, 1981; Li et al., 1981; Milnor, 1990; Nichols and O'Rourke, 1990).

Wave summation effects can either increase (constructive interference) or decrease (destructive interference) pressure pulsatility. Thus, their interactions affect the opposition to blood flow which is presented to the heart by the systemic vasculature (systemic input impedance). Since a given blood volume ejected against a high pressure requires more energy per stroke than the same volume ejected at a lower pressure, it is advantageous for the pulse pressure to be minimized at the heart. The amplitude of reflected wave elements and the timing of their return to the heart is therefore critical to the determination of systemic input impedance (Porje, 1946; McDonald and Attinger, 1964; Elzinga and Westerhof, 1973; Westerhof et al., 1972; O’Rourke, 1982; Fitchett, 1991; O’Rourke et al., 1992). Indeed, investigators have demonstrated that impedance is determined by the length of the arterial tree (L), the pressure pulse wave velocity (C) and the frequency of cardiac contraction ($f_0$) (Noordergraaf, 1978: Noordergraaf, Li and Campbell, 1979; Milnor and Nichols, 1975; Milnor, 1979; O’Rourke and Yaginuma, 1984). When the incident wave transit time through the arterial tree is
long in comparison with the duration of the cardiac cycle the
interactions with its reflected components are such that the
input impedance is reduced and peripheral pressure is augmented.
In contrast, if C is high, L is short, or ventricular ejection
time is protracted (slow heart rate), the reflected waves may not
significantly effect systemic input impedance or the pressure
pulse profile.

The interactions between incident and reflected wave elements are
commonly described by either of two principal models, the
Windkessel and the Tubular, (Aperia, 1940; Womersley, 1957b;
Burkhoff, 1988; Nichols and O’Rourke, 1990; Milnor, 1990;
Fitchett, 1991). In each model the circulatory system is regarded
as a system of distensible tubes through which pressure and flow
waves are propagated and in which the velocity of wave
propagation is determined by arterial viscoelasticity and blood
density. The Windkessel theory interprets the arterial system as
a single elastic reservoir which at one end accepts an
intermittent delivery of blood and at its numerous terminations
delivers a steady stream to the resistance vessels. The arterial
circulation thus functions as both a cushion and a conduit where
pressure fluctuations created by intermittent ventricular
contraction are smoothed (cushioned) and blood is delivered to
the peripheral tissues with a minimal decline in mean peripheral
pressure (conduit). In this model C is infinite, hence wave
reflection is not possible and, as a result, pressure and flow
pulses in the central arteries change simultaneously (Burkoff et
In contrast, the "Tubular" model is characterized by a finite wave velocity where the incident wave and its reflected components interact to augment peripheral pressure while minimizing systemic input impedance. In this model the wave element interactions create an oscillating pressure envelope along the length of the arterial tree which is then, in effect, punctuated by a series of nodal and antinodal points. When the correlation between \( L \) and pressure pulse wavelength (\( \lambda \), determined by \( C / f_0 \)) is optimal, the heart is functionally located at a node approximately one-quarter \( \lambda \) back from the effective peripheral reflecting site (the sum of reflection effects in the lower body of most mammals is thought to be realized at the iliac bifurcation). Hence, constructive summation effects augment peripheral pulsatile pressure while the heart is uncoupled from the high terminal impedance of the arterioles. (O'Rourke and Taylor, 1967; Milnor, 1979; Noordergraaf et al., 1979; O'Rourke and Avolio, 1980; Li, 1983; O'Rourke et al., 1984, 1989; Fitchett, 1991; Li and Noordergraaf, 1991).

In animals whose metabolic requirements are low, simple arterial recoil provides sufficient force to maintain mean arterial and pulsatile pressures and flow to the peripheral tissues (Jones and Shelton, 1972; Gibbons and Shadwick, 1991; Jones, 1991). In these animals, the \( C / L / f_0 \) relationships required to minimize input impedance through wave reflection effects need not be maintained
and thus, do not limit biological design. Studies in poikilothermic vertebrates have shown that the combination of low $f_0$'s and short L's (long $\lambda$'s) produce pulse wave transit times which occupy a very small portion of the cardiac cycle so that the incident pressure wave traverses the system many times within a single heart beat (Jones and Shelton, 1972; Langille and Jones, 1977; Gosline and Shadwick, 1982; Gibbons and Shadwick, 1991). This series of multiple reflections promotes substantial interactions between components thus, the individual constituent waves become fused, and there is no discrete effect of incident and reflected wave interactions on systemic input impedance. While wave reflections exist, their effects are minimal, and the animals behave as functional Windkessels (Jones and Shelton, 1972; Langille and Jones, 1977; Gibbons and Shadwick, 1991).

Reliance upon arterial recoil alone limits capillary flow rate and the control of cardiac output distribution in part because the pulsatile pressure is reduced by frictional interactions between the blood and arterial walls during travel through the arterial tree. In many mammals, the biological needs imposed by homeothermy and increased hydrostatic force resulting from greater size dictate the need for higher peripheral pressures to drive arteriolar flow. A reduction in arterial distensibility can raise mean arterial pressure, but pressure pulsatility is still reduced during travel and the increased wall stiffness increases $C$. In order to augment peripheral pressure and increase arteriolar flow while maintaining the beneficial correlation between $C$, L and $f_0$, $L$ and $f_0$ must change proportionally in these
animals (Bergel, 1973; Elzinga and Westerhof, 1973; Iberall, 1979; Milnor, 1979; O'Rourke, 1982; Callaghan et al., 1984; Yin, 1987). Milnor (1979) hypothesized that natural selection would favor species whose L’s and f_0’s were matched to minimize cardiac work (assuming that C is approximately the same in different sizes of animals). In very small species the design alterations required to produce this optimal relationship may not be the deciding factor which dictates the mechanisms of cardiac efficiency. Since increases in heart rate also increase myocardial oxygen consumption (CVO_2) the high f_0’s required to match L and create a beneficial L/\lambda correlation may be metabolically disadvantageous. In this case, the higher CVO_2 may outweigh the potential benefits of wave reflection interactions at the input to the systemic vasculature, and stroke work may be minimized primarily by arterial recoil.

While much is known about the circulatory properties of larger mammals, there is little information regarding mature terrestrial species smaller than about one-half kilogram (Aperia, 1940; Attinger et al., 1966; Elzinga and Westerhof, 1973; Avolio, 1976; Milnor, 1979; Li, 1983; Li et al., 1981; Li and Noordergraaf, 1991). Since L declines as body size decreases, C has been demonstrated to be independent of mass, and since the upper limits of f_0 are influenced by both the electrical and mechanical properties of the heart, it is possible that there is a critical point at which the optimal correlation between L and f_0 cannot be realized. While Avolio (1976) reported a C of 4.1 m/s in the descending aorta of guinea pigs, and Milnor (1979) related a C of
approximately 4 m/s in the rat, a velocity equal to that recorded in larger species, both researchers identified only minimal wave reflection effects. The characteristic alterations of the arterial pulse profile and systemic input impedance caused by wave reflections did not appear to be as great as those observed in larger species nor was an optimization of L and λ demonstrated. The combination of short arterial tree length and low heart rates in these two species appeared to prevent the optimal correlation of the heart with a minimum point of pressure and impedance.

Utilizing the principles of allometry, first conceptualized by Max Rubner in 1883, estimations of alterations in physiological function in relation to changes in body mass can be made. This exponential formulation quantifies the function/mass relationship as $Y = aX^b$, where $b$ is an empirically determined exponent expressing the rate of change (Schmidt-Nielsen, 1984). This formulation is often used to estimate alterations in cardiovascular variables for animals outside of an easily measured range. Numerous investigators have provided empirical data which suggest that L decreases with size as a power of 0.33 while $f_o$ changes with mass as a power of -0.25 to -0.32 (Brody, 1934; Stahl, 1965; McMahon, 1973; Milnor, 1979; Spatz, 1991). If a power of -0.32 is used to predict the maximum $f_o$ of a small animal whose mass is 0.040 kg, the resting rate would be 790 beats/min and aortic length would be 0.035 m. Assuming a C of approximately 4.4 m/sec (based on the average C of animals ranging in mass from 1.0 to 200 kg reported by Noordergraaf, Li
and Campbell, 1979) the $\lambda$ would be 0.3341 meters per beat. This wavelength is approximately ten times longer than the estimated $L$ and thus, the pulse would traverse the aorta many times within a single cycle. The resulting interactions between the incident and reflected wave components would be substantial and create a merged wave. Unless $f_0$ was much faster than predicted, or $C$ was much slower, discrete wave reflection effects would probably not be visible in the arterial pulse profile, and there would be little impact on systemic input impedance. As a result, despite the existence of wave reflections, the system would be a functional Windkessel.

Since rats and mice are frequently utilized as models of the human cardiovascular system, and the hemodynamics of these species have not been described, it is of value to determine their circulatory mechanisms. Accordingly, this study was conducted to test the hypothesis that very small mammals are not likely to benefit from wave transmission effects but instead may be characterized as functional Windkessels due to the combination of short $L$'s and inappropriately low $f_0$'s. Consequently, cardiovascular variables were measured to identify the fundamental relationships of $L$, $f_0$ and $C$ in rats, which have been known to display minimal transmission characteristics, and in mice which have not been previously studied. Peripheral vascular resistance ($R_s$) and $f_0$ were altered to assess the effects of these variables on wave reflection characteristics and because $C$ is determined by the viscoelastic characteristics of the arterial tree, the biomechanical features of the abdominal aorta were...
quantified to provide a basis for cross-species comparison. Lastly, empirical measurements were compared with allometrically calculated values to evaluate the accuracy of cardiovascular estimations at the mass extremes.
Chapter I.

The characteristics of circulatory hemodynamics are defined by the functional integration of the systemic vasculature and the heart. While the shape of the flow pulse is determined by both cardiac and arterial mechanical properties, pressure pulse wave features are influenced by the correlation between arterial length (L), the cardiac period (f₀), the velocity of pressure pulse wave propagation (C), and the peripheral resistance (Rs). The interactions of the incident pressure wave generated by ventricular contraction, and its reflected components which originate at points of impedance mismatch, define the pressure pulse profile at any location within the arterial tree. These empirically determined interactions, described mathematically, form the basis of two primary theories of hemodynamics, the Windkessel and the Tubular models. In many mammals, the Tubular theory of hemodynamics describes the effects of incident and reflected wave component interaction on blood flow opposition. In some non-mammalian species, although there are significant wave element interactions, they are not discrete and do not disconnect the heart from the terminal impedance (Jones and Shelton, 1972; Gibbons and Shadwick, 1989; Gibbons and Shadwick, 1991; Jones, 1991). While these species cannot be truly described by the Windkessel model, in which wave reflections do not exist, they are aptly represented as functional Windkessels.
SECTION 1: THE ARTERIAL SYSTEM

ARTERIAL ANATOMICAL PROPERTIES

In mammals, all arteries display a common pattern of organization and are composed of four primary tissue types whose proportions vary with location in the circulation. The arterial wall is demarcated by three concentric regions: the tunicas intima, media, and adventitia (Bergel, 1961; Fung, 1968). The tunica intima has two components; the endothelium, a single cell layer which lines the internal surface of the vessel and which is surrounded by a thin subendothelial layer containing a few fibroblasts and collagen fibers, and the internal elastic lamina, a layer of branching elastic fibers that form the inner boundary of the next layer.

The second layer, the tunica media, is usually the thickest portion of the wall. It shows the most variation in structure and properties at different locations within the vascular tree. In the large central arteries it is composed of multiple concentric layers of elastic tissue, collagen fibers and smooth muscle cells which cross-link the successive elastic layers. The smooth muscle cells are thin and contain contractile filaments which constrict and generate tension in response to depolarization of the cell membrane. In the large arteries, the cell orientation is normally oblique or longitudinal, while in more peripheral arteries it is generally circumferential and forms flat spirals. Peripherally, the media consists largely of spirally arranged smooth muscle cells which are concentrated in multiple layers with small
amounts of connective tissue between them. The number of these layers normally decrease as vessel radius diminishes, but in all arteries the tunica media is the predominant element in the wall.

The outermost layer of the arterial wall is the tunica adventitia, and this layer may be as thick as the media in some locations. It is less prominent because it is made up of loose connective tissue built from elastin and collagen fibers, which lie predominantly longitudinally, and at most locations the outermost edge is ill-defined. In arteries greater than about 1 mm in diameter the adventitia is supplied by the vasa vasorum, a network of blood vessels which supply nutrients to the tissue. In very small vessels, nourishment of the tunicas intima and media depend on transport of materials from the lumen. In all arterial vessels, the tunica adventitia is also infiltrated by small lymphatic vessels and nerve fibers which run to the smooth muscle cells. (Peterson et al., 1960; Bergel, 1961; Haynes, 1963; Cox, 1978; Fung, 1968, 1981; Gosline, 1991).

ARTERIAL BIOMECHANICAL PROPERTIES
Classical elastic theory is often used to describe materials according to their ability to deform in response to an applied force (stress) and their tendency to return to their original shape when the stress is removed. Robert Hooke (1678) provided a first understanding of elasticity from experiments which demonstrated the stress-strain proportionality of metal wires which he described mathematically (Hooke's Law) (Wolinsky and Glagov, 1964). Later, other investigators including Thomas Young
(1773-1829), improved the precision of Hooke's law through demonstrations of wall tension/cross-sectional area proportionality with regards to strain in identical materials. Young's experiments defined the static range dependance of linear elasticity (strain effectively reduces to zero when the applied stress is reduced to zero) and from these he postulated a descriptive value, \( E \) (Young's modulus of elasticity). This value increases, within a known range of stresses, as the force per unit area required to deform the material increases. The calculation of \( E \) assumes both homogeneity (uniformity in composition) and isotropy (\( E \) is independent of the direction of the applied stress) of the medium (Peterson et al., 1960; Caro et al., 1978).

While most biological materials are neither homogeneous nor isotropic and are therefore not truly elastic, many are considered visco-elastic. These materials demonstrate traits such as creep (a gradual rise in strain with applied stress accompanied by a dissipation of mechanical energy) and stress-relaxation (the stress required to maintain a given strain decreases slowly over time) (Patel and Fry, 1964; Bergel, 1973; Posey and Geddes, 1973; Cox, 1975). Arterial walls, composed of a mesh-like network of elastin, collagen and smooth muscle fibers, are visco-elastic in nature, and the wall properties are determined by a combination of the behavior of the individual components. As such, the alterations in shape and size displayed in response to the pressures and shear stresses exerted by the blood and the restrictions imposed by the surrounding tissues
cannot be defined by a single Young’s modulus. But E is very informative if a range of known stresses is applied to an arterial segment, and from those measurements E is defined as if the wall were both homogenous and isotropic. In this manner, all the components of the arterial wall are lumped together and the behavior can be described by an effective E (E_{eff}). When E_{eff} is measured for a small deformation at a known stress, the value is defined as the incremental Young’s modulus (E_{inc}). This measurement is a useful parameter for direct comparison of different vessel segments tested under identical conditions. Since arteries are capable of stretch in both the longitudinal and circumferential directions, in vivo examination is particularly informative because tethering to surrounding tissues has been shown to inhibit longitudinal wall motions more than radial ones (Bergel, 1961; Callaghan et al., 1984, 1986). Hence, when E_{inc} is calculated in vivo from pressure dependent diameter changes about the species-specific physiological pressure value, longitudinal wall motions can be ignored, and direct comparisons of cross-species biomechanical properties can be made (Posey and Geddes, 1973; Shadwick and Gosline, 1981, 1985; Gosline, 1991).

The relative distribution of elastin, collagen and smooth muscle tissue varies with location within the arterial tree of a single animal. In mammals, the central vessels, primarily the ascending, descending and thoracic aortic segments, have a greater proportion of elastin than the peripheral vessels (Hamilton, 1963; Cox, 1975; Guyton, 1981). The value of E_{inc} generally rises with increasing distance from the heart, indicating that
Peripheral vessels are stiffer than the proximal arteries, and in all locations there is a rapid increase in vessel wall stiffness as the radial distension increases. Upon inflation, the arrangement of the collagen and elastin fibers changes so that at low strains most collagen fibers are slack and the majority of the stress is borne by elastin, while at higher strains the collagen fibers straighten and take up stress. This property stiffens the wall as the stretching force increases.

Properties of Blood and the Effects of Viscosity

The fluid medium of the circulatory system (blood) consists of a suspension of a variety of cells in an aqueous medium (plasma) of relative uniformity. The hematocrit (volume percentage of cells to plasma) has been shown to be consistent across many mammalian species at approximately 45% for normal blood. Whole blood density is between 1.05 and 1.06 gm/cm³ and remains constant across the range of temperatures and pressures experienced in the circulatory system (Taylor, 1959; Guyton, 1981; Milnor, 1990).

Viscosity (a measure of frictional resistance which results from two surfaces sliding past each other) can significantly influence flow properties. In the circulatory system, fluid behavior is often described in terms of its "Newtonian" properties, a condition in which the apparent viscosity is constant at all rates of shear. While Newtonian flow is approximated by homogenous fluids, suspensions of particles normally show deviations from this behavior so that the apparent viscosity increases with decreasing shear rates. In blood, numerous
investigations have demonstrated that the non-Newtonian effects of flow are most prominent in very small diameter vessels where cell and tube dimensions are nearly equal (Taylor, 1959; Haynes, 1963; O’Rourke and Taylor, 1967). Indeed, both Taylor (1959) and Haynes (1963) have shown that in the large central arteries, the non-Newtonian effects on wave transmission and flow properties are small. In addition, Haynes and McDonald (in Nichols and O’Rourke, 1990), have demonstrated that the normal shear values in the circulation are too high for small changes in viscosity to be significant and that blood flow in the larger arteries may be considered to be Newtonian in nature. This assumption indicates that blood may be considered to be a homogenous fluid and its velocity is the same at all rates of shear (Li, 1988).

PRESSURE WAVE PROPAGATION AND THE DETERMINATION OF WAVE-SPEED
As the left ventricle contracts, blood is ejected into the entrance of the aorta causing an increase in blood pressure and distension of the vessel wall. As cardiac ejection slows, pressure begins to decline, and the expanded wall recoils to its equilibrium position. The inertia of the blood keeps it moving forward even after the driving pressure difference has decreased, and as a result, the first piece of the artery overshoots its equilibrium position. This creates an oscillatory motion and the wall disturbance is passed to the next section of the wall which also distends. As that section recoils, the cycle is repeated and the disturbance is thus propagated along the arterial tree as a pressure wave. Although the pressure wave can travel in either direction, in systemic arteries it primarily begins at the heart.
and propagates distally (Milnor and Bertram, 1978; Li et al., 1981, 1988; Salotto et al., 1986).

The velocity of pressure pulse wave propagation \( C \) (m s\(^{-1}\)) within the arterial tree is governed by the balance between the inertia supplied by the mass of the blood (characterized by the blood density \( \rho \) (kg m\(^{-3}\)), and the restoring force of the arterial walls. If the walls are highly distensible the wave velocity will be slow, whereas an increase in wall stiffness increases \( C \) (Anliker et al., 1968; van Loon et al., 1977; O’Rourke and Brunner, 1992). The distensibility \( D \) (m\(^2\) N\(^{-1}\)) of the vessel wall, a measure of the fractional change in cross-sectional area in relation to a small change in pressure, has been shown by detailed mathematical analysis to be proportionally related to \( C \) as 1) \( C = \left( \rho D \right)^{-1/2} \) where \( D \) can be expressed in terms of \( E \) according to the equation 2) \( \left[ 1 / E(N \text{ m}^{-2})(h \text{ (m)} / d \text{ (m)}) \right] \) and \( h/d \) is the wall thickness to diameter ratio (Caro et al., 1978). Substitution of these two equations then allows \( C_0 \) (the predicted velocity) to be calculated according to the Moens-Korteweg (1878) equation as 3) \( C_0 \) (m s\(^{-1}\)) = \( \left[ (E \text{ (N m}^{-2}) \text{ (h(m)} / \rho \text{ (kg m}^{-3}) \text{ d(m)} \right]^{1/2} \). This simplified model of arterial pulse wave propagation, based on two fundamental principles of fluid flow (conservation of mass and Newton’s second law) assumes that the blood viscosity has no effect on motion and that the pressure pulse disturbance is small enough for the elastic wall properties and fluid mechanics to be linear i.e., the wall behaves like a thin, homogeneous membrane.
As was discussed earlier, the effects of fluid viscosity are small and are likely to lead to only a very small error in the calculation of $C_o$ in large arteries (Caro et al., 1978; Li et al., 1981). But the assumption of linearity implies that for a given frequency a single oscillation in pressure is directly associated with a single oscillation in flow velocity. In large arteries this is approximately satisfied in that 1) while the alterations in diameter with regard to fluctuations in pressure create both local and convective accelerations, there are only small variations in fluid velocity along the vessel length and 2) the time required for significant changes in local fluid velocity is small compared with the time taken for the fluid element to be convected.

Research has shown that these assumptions are applicable to predictions of $C_o$ if the pulse pressure is small compared with the mean pressure (for most arteries the ratio is normally about $0 \leq 2$) (Caro, 1978; Li et al., 1981, 1988, 1991). Comparison of the values of $C_o$ measured empirically from simultaneous recordings of the pressure pulse at two locations in the arterial tree separated by a known distance, and $C_o$ have been demonstrated to differ by no more than 15% (Caro et al., 1978). This difference is small and within the range of experimental error. The Moens-Korteweg equation demonstrates that the inaccuracy of predicted wave-speed calculations introduced by the assumptions regarding viscosity, elasticity, and linearity are essentially inconsequential. If $E_{inc}$ is carefully determined, the pressure pulse wave-speed can be predicted quite accurately from arterial
biomechanical properties.

WAVE TRANSMISSION AND REFLECTION

Every point in the arterial tree which is distinguished by alterations in wall properties or branching is a source of partial reflection of the incident pressure wave, with the strongest reflections occurring at junctions between arteries (Aperia, 1940; Haynes, 1963; Anliker, 1968; Milnor and Nichols, 1975; Busse et al., 1978; Avolio et al., 1983, 1984; O'Rourke and Yaginuma, 1984). When a wave travels down a single artery (parent) and encounters a junction (daughters), a portion of the wave travels back up the parent (reflected) and additional portions are transmitted down the daughters. If the daughter arteries have the same E and distensibility as the parent, and the sum of their cross-sectional areas are equal to the parent, the opposition to blood flow (impedance) will be the same in each artery. In this instance, there is little energy in the reflected wave component, and it does not significantly affect the pressure pulse in the parent artery. If these conditions are not met and there is an impedance mismatch, the energy of the reflected component is quite large, and as it travels back toward the heart it alters the shape of the pressure pulse. In conditions of impedance mismatch, the reflected wave can be either positive or negative, but in most cases the arteries behave as if they were a closed end system. The phase of the reflected component of the pressure wave is therefore unchanged while the reflected flow wave is out of phase (negative) in relation to the incident flow wave. This results in a reduction in the oscillatory component of
flow and an increase in the amplitude of the pressure oscillation at the site of the impedance mismatch.

In most vertebrates, the number of branches and the rate of decline in vessel radii are most prominent distal to the iliac bifurcation of the abdominal aorta. As a result, the peripheral arterioles present the greatest opposition to blood flow in the lower body. The reflective components of the incident wave which travel back toward the heart from the peripheral vasculature appear to meet at the iliac bifurcation and, as such, it is at this point that the sum of the terminal impedance is realized. Most investigators agree that the effective length of the aorta in vertebrates can be measured from the root of the ascending aorta to the iliac bifurcation (Campbell et al., 1989; Gibbons and Shadwick, 1989; Gosline, 1991).

PRESSURE PULSE WAVE CONTOUR

While the arterial pressure wave profile is dependent upon multiple factors including mean arterial pressure (MAP), systemic arterial vasomotor tone, the duration of ventricular ejection, and C, it is the presence of wave reflections in the systemic arterial tree which most affects wave shape (McDonald and Attinger, 1964; Avolio and O'Rourke, 1986). When blood is ejected into the aorta, pressure rises rapidly and is seen in the pulse curve as a steep increase from the foot of the pulse (the anacrotic limb) (figure 1,a). Since blood enters the aorta more rapidly than it exits through the arterioles, due to the inertial resistance of the blood to acceleration, the central arterial
Figure 1. Mammalian pressure waveform (reproduced from Patel and Fry, 1963).

H₂O PRESSURE

A Anacrotic Limb
B Dicrotic Limb
C Incisura
D Dicrotic Wave

--- 0.5 sec. ---
blood volume increases temporarily. This increased volume results in a short period of high pressure which falls as blood continues to flow through the peripheral vessels. This pressure decrease is visible as the descending (dicrotic) limb of the pulse (figure 1,b). When the ventricular pressure falls below that in the aorta, the aortic valve closes and the dicrotic notch, or incisura, is seen in the pressure pulse tracing (figure 1,c). After the closure of the aortic valve, there is a slight upswing of pressure (the dicrotic wave) (figure 1,d) followed by a gradual decrease in aortic pressure as the runoff into the peripheral circulation continues. Alterations in the amplitude of the dicrotic wave as well as fluctuations in wave shape are determined by the timing of the return of peripheral wave reflections (McDonald and Attinger, 1964; Milnor, 1990; Nichols and O’Rourke, 1990; O’Rourke et al., 1992).

THE CONTOUR OF FLOW WAVES

The contour of the flow wave is determined by both cardiac properties and the physical attributes of the arterial tree in mammals. While the distensibility of the arterial walls affects the local pressure gradient as the pulse-wave travels, its influence on flow rates and velocity profiles is negligible. Since the wavelength is very long in comparison with the distance travelled by an individual fluid element during a single pulse, the segment of artery traversed remains approximately uniform in area, thus the segmental flow velocity is not altered.
Comparison of ascending aortic arch flow patterns across a wide range of mammalian species demonstrates very similar waveform profiles (figure 2). Forward blood flow begins in the ascending aorta when the aortic valve opens during ventricular contraction. The velocity rises rapidly to a peak and then, more slowly, falls again. A brief period of backflow is normally observed when the aortic valve closes, and this is followed by almost complete cessation of blood flow for the remainder of the cycle. Investigations have shown that under normal resting physiological conditions, forward flow occupies the first one-quarter to one-third of the cardiac cycle (Aperia, 1940; Hamilton, 1963; Attinger et al., 1966; Elzinga and Westerhof, 1973; Nichols and O'Rourke, 1990; Fitchett, 1991). When heart beat frequency increases, the duration of ejection increases relative to the diastolic period. Thus, forward flow occupies a greater portion of the cycle and the diastolic fill time is decreased (Wetterer, 1954; Attinger et al, 1966; O'Rourke, 1967, 1992; Elzinga and Westerhof, 1973; Busse, et al., 1975).

INDICATORS OF CARDIAC WORKLOAD
Since the heart is a pump which converts chemical into mechanical energy, its function can be described by physical principles. The energy required for contraction is quantified through measurements of myocardial oxygen consumption and is comprised of three elements under resting conditions: external mechanical work, tissue metabolism, and potential mechanical energy (Hamilton, 1963; Haynes, 1963; Milnor, 1990).
Figure 2. Cross species comparison of ascending aortic flow patterns (reproduced from O'Rourke, 1982).
Tissue metabolism is a measure of the energy which is required to maintain the metabolic processes of the cardiac tissues. Potential mechanical energy is defined by the pressure that remains in the ventricle at the end of ejection which is, under normal circumstances, dissipated as heat during the isovolumic relaxation phase of the cardiac cycle. External mechanical work is a measure of the energy imparted to the blood during ventricular contraction. This energy expenditure can be quantified from measurements of blood pressure and flow throughout the ejection period. The rate of doing work, or ventricular power, is equal to the product of pressure and flow at all instants during that time (Sarnoff and Mitchell, 1962; Milnor, 1990; Nichols and O'Rourke, 1990).

Cardiac efficiency, as in any machine which expends energy, is defined by the ratio of the power output to the total energy used (the ratio of cardiac work to oxygen consumption). The work of the heart depends on the various arterial factors which combine to oppose the motion of blood and the additional workload imposed by the pulsatile nature of the circulatory system. As such, there are two components to blood pressure and flow: the steady mean value which depends largely upon the peripheral resistance, and the pulsations around the mean value which are determined by the viscoelasticity of the central arteries and the wave reflections present in the system (Sarnoff and Mitchell, 1962). Since a given stroke volume ejected at a high pressure requires a higher oxygen consumption per unit of external work than the same stroke volume at a lower pressure, the reduction of pulsatile pressure
fluctuations at the input to the systemic vasculature is central to the minimization of cardiac workload. An optimal correlation between $L$, $C$, and $f_0$ promotes this reduction by effectively locating the heart at a node of pressure and impedance.

**SECTION 2: HEMODYNAMIC MODELING**

**THE CONCEPT OF VASCULAR IMPEDANCE**

Cardiovascular impedance, a measure of the opposition to pulsatile blood flow presented by the circulatory system, is analogous to the conventional use of the same term in electric current theory. This term, confined to oscillatory motion or alternating current, differs from "resistance" which is a measure of motion in non-oscillatory, steady-state conditions (Nichols and O'Rourke, 1990). The theoretical concept of impedance is based on mathematical principles which describe, through a series of linear equations, the relationship between the pulsatile elements of arterial pressure and flow at each component frequency of the wave (Womersley, 1957b).

*Input impedance* is defined by a frequency spectrum of the ratio of pressure and flow recorded at a particular arterial site, and is indicative of the input to all the vascular segments beyond the point of measurement. When these recordings are made in the ascending aorta of an animal, the impedance values reflect the blood flow opposition of the entire arterial tree. The amplitude of the pressure/flow ratios identify the summation effects of
incident and reflected wave components and thus, the magnitude of the hydraulic load presented to the left ventricle of the heart (Elzinga and Westerhof, 1973; Fitchett, 1991). In this instance, the impedance value is referred to as systemic input impedance.

The opposition to flow in the peripheral vascular bed, measured immediately upstream from its termination (usually at the iliac bifurcation in mammals), is termed the terminal impedance. This value is regarded as a representation of the opposition to flow afforded by the high-resistance arterioles. Investigations have shown that the properties of the arterioles are almost completely resistive, and thus, terminal impedance is usually considered in terms of mean pressure divided by mean flow (Nichols and O'Rourke, 1990; O'Rourke et al., 1993). Although the terminal impedance is not identical to the calculated systemic peripheral resistance, in physiologically normal animals it is only slightly less. Calculations of peripheral resistance are therefore, usually taken to be representative of the terminal impedance.

**CALCULATING VASCULAR IMPEDANCE: FOURIER ANALYSIS OF WAVEFORMS**

The analysis of pulsatile fluid flow can be quantified by the use of *Fourier Series* analysis, a mathematical degradation of complex pressure and flow pulse waves into their sinusoidal, or harmonic, components (Aperia, 1940; Porje, 1946; Womersley, 1957b; Nichols and O’Rourke, 1990). This mathematical principle may be applied to any system which is both linear and is a time-varying periodic function. Under these conditions the harmonics each have different amplitudes and frequencies, are out of phase with each
other and their superimposition reproduces the original waveform. If the system is non-linear, the different harmonics will affect each other and the behavior of the composite wave cannot be described through the summation of the separate harmonics (Blackman and Tukey, 1959; Chatfield, 1989). The harmonic frequency is dictated by the fundamental (or repetition) frequency of the wave itself and is designated as the first harmonic. Each subsequent harmonic has a frequency which is an integral multiple of the fundamental. Aortic pressure and flow velocity waveforms can be accurately described by a constant term (the mean level about which the pulsatile component fluctuates) plus the first 10 harmonics. However, 95% of the wave energy has been shown to be within the first five to seven harmonics in each waveform (Caro, 1978; Nichols and O'Rourke, 1990). In the pressure waveform, the mean term is the largest in amplitude and the small higher frequency terms describe the sharp fluctuations of the dicrotic notch. In contrast, the flow velocity waveforms are characterized by a smaller mean term and a larger fundamental amplitude. The higher frequencies in this wave describe the rapid early systolic rise in flow and the sharp fluctuations of the end-systolic flow reversal.

The analytical requirements of linearity and periodicity must be considered with regards to the use of Fourier analysis in the circulatory system. Investigators have shown that although the arterial system is non-linear in the precise definition of the term, the predicted non-linearities are normally not greater than the order of accuracy in the instrumentation used to obtain the
measurements. Any arterial non-linearities are therefore impossible to detect and are considered to be empirically inconsequential (O’Rourke and Taylor, 1967; Taylor, 1973; Caro, 1968; Fung, 1981). Despite the rhythmicity of the heart beat, there are fluctuations associated with the respiratory cycle and unpredictable ectopic beats. The respiratory effects cause a sinus arrhythmia, and a fluctuation in MAP, as a result of changes in both intrapleural pressure and circulatory reflexes. In addition, Traube-Hering waves, resulting from oscillations of the central nervous system mechanisms of pressure control, are superimposed upon these respiratory fluctuations (Sarnoff, 1962; Haynes, 1963). Since, as was previously stated, Fourier analysis requires periodicity within the system, this introduces additional concerns regarding the validity of its use in the cardiovascular system.

Research conducted in dogs has shown that the time required to achieve steady-state oscillation (true periodicity) in the arterial system is normally less than one heart beat (Taylor, 1964; McDonald and Attinger, 1964). Taylor (1965, 1966a, 1966b) performed qualitative examinations of the periodicity of the heart beat in dogs whose hearts were either beating normally or were paced to irregular rhythms. His findings were analyzed with a correlation (or power distribution) analysis. The results indicated that, under normal conditions, slight variations in pulse frequency introduce little error into the harmonic analysis of pulse trains. Thus, the requirement of periodicity is met. In addition, many researchers have demonstrated the validity of
Fourier analysis by accurately reproducing pulsatile waveforms from their harmonic components (for review, see Nichols and O’Rourke, 1990). As was discussed, comparison of the statistical variance of a wave and its Fourier spectrum demonstrates that 99.5 per cent of the energy is included in the first five to seven harmonics, and generally, no appreciable variation is obvious by adding additional harmonic components (Attinger et al., 1966; Taylor, 1973; Nichols and O’Rourke, 1990).

THE WINDKESSEL THEORY
The time-varying changes of blood flow and pressure were first described by Stephen Hales in the mid 1700’s who compared the arterial system’s function to the inverted air-filled dome of the fashionable Windkessel fire engine (Saksena, 1983). Hale’s early description later prompted the two-element Windkessel theory, proposed by the German physiologist Otto Frank in 1899. In Frank’s model, the arterial system is portrayed as a single elastic reservoir (the compliant central vessels) linked to a single resistance element (the peripheral vasculature). Since Frank’s original theory there have been several modifications, including an expanded three-element model, which utilizes a characteristic impedance component in addition to the arterial compliance/peripheral resistance configuration (Westerhof et al., 1969; Yin, 1987; Burkhoff et al., 1988). In both the two-element and three-element models, the sequence of events is the same: upon contraction of the heart, the systemic arterial vessels are inflated almost simultaneously at all points throughout the tree because the pulse wave velocity is effectively infinite. Pressure
energy, stored in the highly elastic ascending aorta, is then expended as arterial recoil to convert the pulsatile blood surge into a smooth outflow to the periphery (figure 3a).

THE TUBULAR THEORY
Numerous investigators have contributed to the understanding of pulsatile pressure and flow interactions since the early investigations of Frank. New models of pulsatile events have evolved, each of which describe the movement of blood at every point in the system by differential equations of force and motion. The most popular version was developed by J.R. Womersley (1957) and has been used by many investigators as a foundation for their experimental work (McDonald and Attinger, 1964; Taylor, 1964; Attinger et al., 1966; O’Rourke and Taylor, 1967; Bergel, 1973; Avolio, 1976; Nichols and O’Rourke, 1990). This model treats each artery as a string of identical units, linked together, through which pressure and flow waves pass sequentially. It assumes that the pressure changes generated by the heart do not occur simultaneously throughout the arterial tree but instead travel as a wave through the system to arrive at sites distal to the heart. Since the arterial system is considered to be a closed loop containing multiple branching points and sites of change in wall elasticity and radii, pressure and flow waves can be reflected back towards the heart. Studies have shown that despite the numerous points of discontinuity, the primary site of wave reflection appears to be the terminal vascular bed (Attinger et al., 1966; Avolio, 1976; Langille and Jones, 1977; Noordergraaf et al., 1979). The incident wave
Figure 3. Schematic diagram of the Windkessel model (a) and the Tubular model (Wave Transmission concept) (b) (reproduced from McDonald, 1960).

A 'windkessel' model of the circulation. The major arteries are represented by a single elastic chamber with compliance $C$, while the peripheral vasculature is represented by a single resistance $R$. Total flow in systole, $Q_s$, is the sum of the volume change of the elastic chamber, $Q_c$, and the simultaneous flow across $R$, $Q_r$. Flow in diastole ($Q_d$) is caused by the recoil of the stretched walls of the elastic chamber.

A diagram to show the interaction of an incident and a reflected pressure wave at a closed end (terminal vascular beds). For clarity it is assumed that only 40% of the wave is reflected (i.e. reflection coefficient = 0.4). The abscissa is marked in fractions of a wavelength. At the point of reflection both waves are in phase and sum together. With reference to a point one quarter wavelength away, the incident wave is 90° earlier and the reflected wave 90° later so that they are 180° out of phase and cancel. This point is a node and the only oscillation is the difference between the maximum amplitudes of the incident and reflected waves. For maximum benefits in terms of promoting cardiac efficiency the heart should be located at this point. The total excursion throughout the cycle is represented by the wave envelope (heavy outer lines).
produced by cardiac contraction can be either constructively or destructively influenced by its reflected components. As an example, at the terminal end of the arterial system both the incident and reflected wave elements are in phase and sum to produce a resultant wave that is greater in amplitude than the incident alone. As the reflected elements travel toward the heart they move progressively out of phase with the incident components, producing a wave that is smaller in amplitude. The interaction of the wave components thus produces a series of nodes and antinodes throughout the vessel. These interactions seem to be most beneficial when the reflecting site is located one-quarter $\lambda$ from the heart (figure 3b). At this point, the heart is ideally located at a node so wave summation effects can increase peripheral pressure pulsatility without increasing systemic input impedance (Womersley, 1957b; Taylor, 1964, 1965; Westerhof et al., 1972; O’Rourke, 1982; Salotto et al., 1986; Westerhof and Huisman, 1987; Yin, 1987; Jones, 1991).

THE IMPEDANCE SPECTRUM: INDICATORS OF WAVE REFLECTIONS

Based on Womersley’s linearized equations, Taylor utilized tubular models of the circulation to conduct some of the first definitive studies on wave reflection and systemic impedance patterns (Taylor, 1959, 1964, 1965, 1973). Detailed experiments were done by other investigators, and although several different experimental models were utilized, the patterns of impedance in Tubular systems were similar (Wetterer and Kenner, 1971; Noordergraaf, 1978; Westerhof et al., 1969, 1970, 1987; O’Rourke, Kelly, and Avolio, 1992). In general, the impedance spectra
generated by tubular models show a maximum amplitude at the characteristic impedance (zero harmonic) followed by a smaller amplitude at the fundamental frequency. These harmonics are succeeded by amplitude fluctuations such that smaller maxima are observed at successive harmonic frequencies which correspond to approximate one half-wavelength intervals. Minima in impedance moduli are noted at harmonics which fall at one-quarter wavelength multiples, while the phase, which identifies the relative timing between the pressure and flow (flow precedes pressure at the point of measurement when the phase is positive), crosses zero at frequencies which correspond to the minima of impedance (figure 4a).

In functional Windkessel systems, the impedance spectrum falls from a maximum value at the characteristic impedance (zero harmonic) to gradually lower values as the frequency increases. There is little or no fluctuation in the impedance amplitudes of the harmonic components. Concurrently, the phase begins near zero at zero frequency and becomes progressively more negative with increasing frequency (figure 4b). A minimum in impedance value is generally reached above the sixth or seventh harmonic. Thus, the one-quarter wavelength frequency occurs at a point of insignificant contribution to the pulse. These modulus and phase values are indicative of the absence of significant wave propagation and reflection effects (McDonald and Attinger, 1964; Bergel, 1961; Nichols and O'Rourke, 1990).

The majority of in vivo investigations of systemic input
Figure 4. Systemic vascular impedance spectrum and phase of (a) the Tubular model and of (b) the Windkessel model (reproduced from McDonald, 1960).
impedance and left ventricular workload have been conducted in canines (Patel and Fry, 1963; Attinger et al, 1966; O’Rourke, 1982). While some measurements have been made in other larger mammalian species, the impedance results demonstrate very similar patterns to the dog, hence, this species is used as an example (Avolio, 1976; Murgo et al., 1981b; Yaginuma et al., 1986). The general patterns of impedance in the ascending aorta (the input to the systemic vasculature) show that the first minimum of impedance modulus occurs at a lower frequency than in other arteries. Usually, after the first minimum, the impedance modulus either remains low over a wide range of frequencies or rises slightly to fall to a second minimum at close to twice the frequency of the first. At the same time, the impedance phase fluctuates and remains below zero until well above the frequency of the first minimum (Figure 5). These findings are indicative of the cancellation of wave reflection effects from discrete reflecting sites and demonstrate the importance of \( L, C, \) and \( f_0 \) matching to uncouple the left ventricle from the high terminal impedance.

PRESSURE WAVE DISTORTION

Measurements of pressure pulse waves have been made in mammals ranging in size from guinea pigs to horses and, although the wave characteristics are similar in all species, the distortion effects which result from reflected waves do not seem to be as pronounced in small mammals (Avolio, 1976; Noordergraaf and Li, 1979; Zahka et al., 1989). As was previously discussed, in many mammals there is a measurable alteration in both the shape and
Figure 5. Canine systemic aortic input impedance, and femoral artery impedance, modulus and phase spectra (reproduced from Nichols and O'Rourke, 1990).
magnitude of the pressure pulse as it travels toward the periphery. Pressure pulse amplification or "peaking" is greatest at the termination of the abdominal aorta and is observed as an increase in both pulse pressure and rate of rise of the front of the wave (steepening). In addition, there is generally a decline in the amplitude of the dicrotic wave, and the sharp inflection at the incisura becomes smoothed with travel away from the heart (Figure 6). These distortion effects result from wave element interactions determined by vessel wall properties. Since the pulse wave is propagating in a viscous system it is attenuated as it travels toward the periphery. The degree of attenuation is different for each of the various frequency components of the pulse, and damping increases with increasing frequency. In addition, there is a decrease in vessel distensibility in the descending and abdominal aorta. As the vessel increases in stiffness, pulse wave velocity increases; hence, the peak of the wave travels faster than the foot (the point at the end of diastole when the steep rise of the wavefront begins) and lower portions. As a result, the wave front becomes steeper and the maximum pressure reached is higher with increasing distance from the heart (Hansen, 1949; McDonald and Attinger, 1964; Anliker et al., 1968; Callaghan et al., 1986).

In addition to the alterations in pressure waveform which occur as a result of viscous propagation and wall properties, the presence of wave reflections also plays an important role in determining wave propagation behavior. Reflections generally occur at points where the cross-sectional areas of the vessel...
Figure 6. Comparison of pressure pulse waveforms (pressure in mmHg) recorded at multiple sites in the systemic vasculature of the dog (reproduced from McDonald, 1960).
change, at branching points, and at the termination of the arterial tree where resistance to flow and total cross-sectional area change abruptly. In addition, reflections result from the gradual tapering of the vessel segments and variations in vessel wall properties. Harmonic analyses of pressure pulses recorded at various sites of the arterial system in mammals generally indicate an increase in amplitude of the lower harmonics as the pulse moves toward the periphery. As was discussed, viscous damping should be apparent as a decrease in these amplitudes. The discrepancy can be explained by the addition of reflected waves to the incident pulse (McDonald and Attinger, 1964; O'Rourke, 1982; Milnor, 1990).

These alterations in pressure waveform during travel have led to some concern regarding the manner in which the apparent pulse wave velocity (C) should be calculated (Busse et al., 1978; Callaghan et al., 1986). The method most commonly used has been to time the travel of the wave foot over a known distance, based on the principle that the interaction of the incident wave with the reflections it creates is minimal in the early portions (McDonald and Attinger, 1964; O'Rourke, 1982). While this has been demonstrated by many investigators, other studies have illustrated that the wavefront retains its identity as well, but the foot of the wave is not always clear unless the distance over which the wave travels is long (Laszt and Muller, 1952; Anliker et al., 1968). An alternative method was introduced by Patel and Fry (1964) which utilizes the time-derivative of the pressure pulse wave. The transit time of the peak of the derivative is
then taken to be representative of the apparent velocity.

SECTION 3: ALLOMETRIC ESTIMATIONS OF PHYSIOLOGICAL FUNCTION

Systematic alterations in physiological function with changes in body mass have been recognized since Max Rubner first proposed his surface hypothesis in 1883. He suggested that the metabolic rate of endotherms should be proportional to body surface area, which for a given density and unvarying proportions, varies as the 0.67 power of its mass. This is generally true for adult individuals within a species whose alterations in size tend to follow isometric principles. In individuals of varying size belonging to different species body proportions do not change in the same manner, and in 1932, Max Kleiber illustrated, that in this instance, metabolic rates are exponentially related to mass as a power of 0.75.

Since this early work, many investigators have demonstrated that the degree of change in a given physiological or morphological variable can be expressed by the general allometric equation $Y = ax^b$, where $a$ is a species specific proportionality coefficient, $b$ is an empirically determined exponent expressing rate of change and $X$ is body mass (Brody et al., 1934; Stahl, 1965; McMahon, 1973; Schmidt-Nielsen, 1984). Allometric equations are formulated according to regression lines calculated from measured values and, as most physiological variables are measured in species ranging in mass from about 1 to 400 kg., the confidence limits of
such predictions are strongest within this range. While extrapolated values can be useful indicators of general alterations in function, they do not identify the effects of differences in biological design.

**ESTIMATIONS OF CARDIOVASCULAR FUNCTION**

Many allometric formulations of physiological function and anatomical dimensions have been established in the cardiovascular system (for an extensive review, refer to Schmidt-Nielsen, 1984). Morphological formulations, in animals ranging in size from mice to Greenland whales, were reported by Clark (1927). He established exponential values of \( A \propto X^{0.082} \) and \( D \propto X^{0.41} \) for ascending aortic cross-sectional area and diameter, and demonstrated a mass dependent decrease in aortic length of \( L \propto X^{0.33} \).

Most alterations in cardiovascular function have been estimated from allometric formulations based upon regression analysis of data measured in a much more narrow mass range. Utilizing data obtained primarily from species between 20 and 200 kg, Stahl (1967) reported that cardiac output varies with mass as \( CO \propto X^{0.81} \). Cardiac output is known to be directly related to resting metabolism and is met by alterations in both heart rate and stroke output. Holt et al., (1962, in Schmidt-Nielsen, 1984) demonstrated that left ventricular output (stroke volume) in the resting animal is constant at about 43% of the end-diastolic volume. This observation is consistent with reported heart mass and external dimension measurements which show that heart size
is maintained at a constant 6% of body mass (Clark, 1927; Stahl, 1965).

Since maximum stroke volume is largely determined by the physical dimensions of the heart, and mass-specific size is constant, this indicates that cardiac output adjustments may be met primarily by alterations in heart rate. Additional support is found in the frequency of cardiac contraction, which has been shown to change as a power of -0.25, the same power function as that for tissue specific metabolic rate (the reported range extends from -0.25 to -0.32) and for total volume circulatory time, which can be estimated from predictions of blood volume and heart rate (-0.25) (Kaplan et al., 1983; Spatz, 1991). Again, since specific metabolic rate, heart beat frequency, and circulatory time scale vary with the same power, it is possible that the increased specific metabolic rate of a small animal could be met primarily by the increase in heart rate (Sarnoff and Mitchell, 1962; Stahl, 1965).

The hemodynamic implications are quite different. As was previously discussed, MAP and the velocity of pressure pulse wave propagation in mammals have been shown to be independent of mass in mammals between 1 and 400 kg. (Haynes, 1963; Li, 1983; Li and Noordergraaf, 1991). Given the cross-species uniformity of C, the interactions of incident and reflected waves would be determined primarily by L and f₀. If cardiac frequency varies with mass⁻⁰.²⁵ and aortic length varies as mass⁰.³³, the estimated f₀ would be too low to place the heart at a node of pressure and impedance in
the aorta.

The design of the cardiovascular system is assumed to be predicated on the need for metabolic efficiency. If allometric estimations of $f_o$ are correct and are adequate to meet tissue specific metabolic needs of a small animal, it is presumed that an additional increase in heart rate would not be economical. Since myocardial oxygen consumption is directly related to frequency, at any given pressure, the metabolic cost incurred as a result of increasing $f_o$ to a rate appropriate for beneficial wave transmission effects may not outweigh the potential benefits. However, if there are wave reflections present in the circulatory system, and $f_o$ is inappropriately low with respect to $L$, systemic impedance and cardiac workload may be increased as a consequence of the systolic return of wave reflections.

Since the correct matching of $L$ and $f_o$ is also a function of $C$, it may be more metabolically efficient to alter $C$ and delay the return of reflections to the heart. Based on allometric estimations of $L$ and $f_o$, it is hypothesized that the $C$ of small mammals may be slower than that measured in larger species. In very small mammals, cardiac workload could be minimized through the reduction of wave reflection effects as opposed to the correct matching $L$, $f_o$ and $C$. To test the validity of this argument, species were chosen for this study based on the reported values of $L$ and $f_o$, and an assumed $C$ of approximately 4.4 m/s, the average velocity reported for mammals (Milnor, 1979; Noordergraaf, Li and Campbell, 1979). Since previous
investigations have shown that rats demonstrate minimal wave transmission phenomena, this species was chosen to provide a basis for comparison with a much smaller mammal, the mouse (Milnor, 1979). In addition, because earlier studies have shown that body shape can influence reflection characteristics, two rodent species were used so that anatomical proportionality would be similar (Avolio et al., 1982, 1985).
CHAPTER II.

MATERIALS AND METHODS

SPECIES SELECTION
In both rats and mice, potential age, gender and breed related differences were minimized by the use of young adult male CD-1 inbred mice and the Sprague-Dawley derived rat. Individual animals were chosen based on an average body mass of 0.035 kg for mice, and 0.600 kg for rats. The maximum acceptable deviation from mean body mass was defined as ± 10% for each species.

ANIMAL HUSBANDRY
All animals were housed in individual polyethylene cages lined with Crown cellulose virgin paper byproduct (Buckerfields, Vancouver, BC, Canada) in an housing facility maintained at an ambient temperature of 21° - 23° C. Purina Lab Chow (Ralston Purina Inc., St.Louis, Mo) and water were supplied ad libitum. None of the animals were fasted prior to surgery.

ANESTHESIA AND SURGICAL INTERVENTION
Mice received Ketamine HCl (Ketalar, 200 mg/ml, M.T.C. Pharmaceuticals, Cambridge, Ontario, Canada) intraperitoneally at a induction dosage of 175 mg per kg. A surgical plane of anesthesia was demonstrated by loss of deep pain sensation, assessed by the absence of toe pinch reflex, and of blink reflex responses. Supplemental ketamine was administered as needed.
Rats received diazepam (Valium, 5 mg/ml, Hoffman-La Roche Ltd., Mississauga, Ontario, Canada) at a dosage of 4 mg per kg intraperitoneally. Once tranquilization was effected, a second injection of xylazine (Rompun, 20 mg/ml, Bayvet, Chemagro Ltd., Etobicoke, Ontario, Canada) at a dosage of 20mg per kg was administered intraperitoneally. A surgical plane of anesthesia was assessed by the method described above.

In all surgeries, the animal was placed on its back and the hair was removed from the ventral neck, thoracic, and femoral areas with small animal clippers. The animal was then restrained in dorso-ventral recumbency on a padded plexiglass surgical board. A thermotemp rectal probe (Physiotemp, Model BAT-12, Sensortek, Inc. Clifton, NJ, USA) was inserted to monitor the animal's core temperature. External heat was supplied by an electric heating pad placed under the surgical board and an infrared warming lamp suspended above the animal. In both species, the animal's core temperature was maintained at an average of 38 °C (± 1.5 °C) through adjustment of the heat sources.

The surgical areas were aseptically prepared with topical iodine and covered with sterile gauze. A midline tracheostomy was performed and in mice, a trimmed, heat flared 20 gauge angiocath sheath with luerlock fitting (Becton-Dickenson, Rutherford, NJ, USA) was inserted into the trachea. The cannula was secured to both the trachea and the skin of the neck with 4-0 silk pursestring sutures. The endotracheal tube for rats was constructed of polyethylene (PE) tubing (sizes from 140 - 200,
Intramedic, Clay-Adams Div., Becton-Dickenson, Co., Parsippany, NJ, USA) glued with quick setting epoxy to needle hubs of the appropriate gauge (18-20, Becton-Dickenson, Rutherford, NJ, USA). The luerlock fitting of the needle permitted the endotracheal tube to be connected to the inhalation line of the ventilator.

The animals were allowed to breath unassisted through the endotracheal tubes while pressure transducers were implanted in the left carotid and left femoral arteries. Midline incisions were made over the arteries and each artery was blunt dissected under magnification (Zeiss Model 10450 Surgical Microscope, Carl Zeiss, Inc. Germany), ligated distal to flow with 5-0 silk suture (Ethicon, Ltd., Johnson and Johnson, Co., Peterborough, Ontario, Canada), and gently retracted with a mosquito forceps. The distance from the intended point of insertion to the estimated location of the aortic arch (proximal) or the iliac bifurcation (distal) was measured. This length was then marked on the pressure transducer with waterproof ink. A slip suture was placed around the proximal arterial segment, an elliptical incision made in the artery wall, and the transducer inserted and advanced to the predetermined distance. The transducer was then secured by tightening the proximal slip suture to pull the arterial wall in contact with the transducer. A figure eight retention suture was placed around the artery and transducer at the distal ligation.

The small diameter of the murine peripheral veins and the tendency for collapse made insertion of intravenous lines technically impractical. As a result, venous cannulations were
not performed in either species in an effort to preserve consistency in technique. Instead, 5.0% Dextrose in 0.9% sterile saline was administered intra-operatively to the mice (Alpha-Pharmaceuticals, Langley, B.C., Canada) via the intra-arterial cannula housing the micropressure transducer at an approximate dosage of 1.2 mls/hour. The fluids were delivered by slow bolus injections of 0.1 mls every 5 minutes. In the rats, the micropressure transducer was introduced directly into the arterial vessels therefore fluids were administered via an additional right femoral artery cannula. The cannula was constructed of tension stretched polyethylene (PE 20, Intramedic, Clay-Adams Div., Becton-Dickenson, Co., Parsippany, NJ, USA) connected directly to a 26 gauge needle (Becton-Dickenson, Co., Parsippany, NJ, USA). This cannula was inserted into the right femoral artery utilizing the same technique as described above but was only advanced approximately 10 mm into the artery. Rats received 5% Dextrose in 0.9% saline via this cannula at a rate of 3.0 mls/hour (0.25 mls slow bolus injection every 5 minutes).

Upon completion of the pressure transducer placement, the animals were placed on external ventilatory support supplied by a SAR Model 830 Small Animal Ventilator (CWE, Inc., Ardmore, PA, USA). Respiratory rates (RR), tidal volumes (TV) and minute volume (MV) for mice were adjusted around the base values of 130 breaths per minute, 0.25 mls per breath and 32.5 mls per minute as needed by each individual. Rats were adjusted around base values of 80 breaths per minute RR, 1.9 mls per breath TV, and 150 mls per minute MV. A midline thoracotomy was performed and the chest
walls retracted to expose the thoracic vessels. The thymus was
dissected free from the pericardial tissue and moved to the right
side of the thoracic cavity. The aortic arch was exposed and a
pulsed doppler flow velocity piezoelectric crystal was positioned
on the ventral surface. This site was chosen for flow velocity
measurements because the ascending aorta of the mouse was
surgically inaccessible. The short ascending aortic length and
close approximation of the innominate and carotid arteries, as
well as the right atrium, made crystal placement at that site
virtually impossible. After securing the crystal with Vetbond
surgical bonding cement (3M Animal Care Products, St.Paul, MN,
USA), a warmed saline-soaked gauze was placed over the thoracic
cavity to retard fluid loss. A photograph of a surgically
prepared mouse is presented in figure 7.

PRESSURE TRANSDUCER CANNULA PREPARATION
In the rats, Millar micropressure transducers (Model SPR-249,
Millar Instruments, Inc., Houston, TX, USA) were introduced into
the blood vessel by direct cannulation. In mice, the small vessel
size necessitated housing the pressure transducer tip in a sheath
composed of a 15 mm segment of polyethylene tubing (PE 160,
Intramedic, Clay-Adams Div., Becton-Dickenson, Co., Parsippany,
NJ, USA). This was connected to a cannula constructed from a
30 mm piece of tension tapered PE-10 tubing slipped inside a 5mm
section of PE-50. Joints were cuffed with 5 mm segments of
Figure 7. The murine surgical preparation. Intra-arterial Millar micropressure transducers, left carotid and femoral arteries. Pulse doppler flow probe placement, aortic arch, via mid-line thoracotomy.
silicone tubing (A-M Systems, Inc., Everett, WA, USA). The transducer and a blunted 26 gauge needle were inserted through the distal end of the PE-160 housing and the silicone cuffs were compressed with 3-0 silk suture ligations to create a pressure tight seal (figure 8). A 1 ml. syringe filled with degassed sterile saline was connected to the needle, via a 3-way stopcock, and the catheter assembly was filled to capacity. After 24 hours, the catheter was visually inspected and any trapped air removed.

The frequency response of the manometer and cannula assembly was established by pop-test (Hansen, 1949), and a minimum response of 250 Hz was used as selection criteria for catheter implantation. A polyethylene "T" tube was connected to the flared saline filled cannula tip of the micropressure transducer-cannula assembly via a Perfectum Adapter (#6184, Popper and Sons, Inc., New Hyde Park, NY). The opposite side of the T was covered with tightly stretched latex secured with surgical tape. The short arm of the T was connected to an air filled syringe and the apparatus was checked for leaks. Pressure was then applied to the T-piece to form a balloon and the latex was "popped" with a 16 gauge needle. Since it was difficult to obtain a perfect seal with the T, each cannula underwent successive "pop" tests until a minimum of 3 consistent response times were obtained. The average of this value was taken to be the frequency response of the integrated micropressure transducer-cannula system. The damped frequency response ($f_d$) and the damping coefficient ($\beta$) were calculated.
Figure 8. Schematic diagram of Indwelling Millar micropressure transducer/cannula preparation utilized in mice.

- 26 gauge Needle, blunted
- Silicone cuffs - 5 mm
- PE-160, 15 mm
- PE-50, 5 mm
- 3.0 Silk Sutures
- Millar Micropressure Transducer
- 30 mm Tapered PE-10

transducer/cannula preparation utilized in mice.
according to the following equations:

\[ 4a) \ f_d = \frac{1}{T_d} \]

where \( T_d \) is the elapsed time from the first to the second oscillatory trough, and:

\[ 4b) \ \beta = \frac{\Lambda}{(4\pi^2 + \Lambda^2)^{0.5}} \]

where \( \Lambda \) is equal to \( \log_e(d_1/d_2) \), and \( d_1, d_2 \) are the first and second oscillatory peak magnitudes; respectively. A representative "pop" test of the minimum accepted frequency response is presented in figure 9.

**PULSATILE AND MEAN PRESSURE MEASUREMENTS**

High frequency response (natural frequency 35 kHz) Millar indwelling micro pressure transducers (Model SPR-249, Millar Instruments, Inc., Houston, TX, USA) were utilized to obtain pressure recordings in both species. Excitation voltage was supplied by a Model TC4 (Millar Instruments, Inc., Houston, TX, USA) bridge interface unit which was calibrated to both an internal electrical standard and by application of a column of mercury to the manometer to determine the voltage to pressure output (electrical drift less than \( \pm 1\% \) per 24 hours). The response was linear to 250 mmHg in all transducers. Background noise levels were determined from analysis of signals obtained with zero pressure and was less than 0.1 mmHg for all transducers. In impedance calculations, no values determined from harmonic components below 0.1 mmHg were analyzed. In addition, the mean difference in measurement between any two transducers used concurrently was determined by simultaneous pressure
Figure 9. Representative pop test of Millar micropressure transducer/cannula interface preparation utilized in mice. All cannulae met or exceeded a minimum requirement of $f_d = 250$ Hz.

$250$ Hz, $\frac{1}{f_d} = 0.004$ Hz

Pop Test, Millar Micropressure Transducer Interfaced in Cannula Preparation for Mice (SPR 205318) - Damped Frequency ($f_d$):

$\frac{1}{f_d}$
application with a mercury manometer. The mean difference was
determined to be approximately 1% (1.0 mmHg) per 100 mmHg for all
transducer pairs.

The micropressure transducer excitation bridge output was routed
through a preamplifying stage (gain 10x) interfaced with an
oscilloscope (Tectronix, Inc., Beaverton, Oregon, USA), a
Hewlett-Packard reel-to-reel magnetic tape recorder (Model 3002,
Hewlett Packard Inc., Sunnyvale, CA, USA) and to a R.C.
Electronics Computerscope System (R.C. Electronics, Inc., Goleta,
CA, USA). A time delay of approximately 4 msec between signal
input and analog-digital conversion through the computer
interface for the screen display was determined using a sine-wave
generator. As a result, when flow was measured simultaneously
with pressure, it appeared to begin prior to the pressure tracing
because of the longer routing time of the pressure measurement
relative to the flow (2 msec).

In both species, the ascending aortic arch pressure was measured
via cannulation of the left carotid artery. The abdominal aortic
pressure was recorded at the level of the iliac bifurcation via
the left femoral artery. Representative analog tracings of
simultaneously recorded aortic arch and abdominal aortic pressure
waveforms measured under control conditions and during vasoactive
drug administration in mice are shown in figure 10. Analog
tracings of both pressure waveforms recorded simultaneously under
control and treatment conditions in rats are shown in figure 11.
Figure 10. Digitized analog recordings of simultaneous measured aortic arch and abdominal aortic (light line) pressures in the mouse. Top line - control, middle line - vasodilated, bottom line - vasocostricted.
Figure 11. Digitized analog recordings of simultaneously measured aortic arch (heavy line) and abdominal aortic pressures in the rat. Top train - control, middle train - vasoconstricted, bottom train - vasodilated.
Representative digitized pressure waveforms recorded in both rats and mice are presented in figure 12.

PULSATILE AND MEAN FLOW MEASUREMENTS

Flow velocities, both instantaneous and mean, were measured with a Pulsed Doppler Flow Meter (Model 545-C, Bioengineering, University of Iowa). This device has a fixed output of 0.5 volts per kHz frequency shift and a frequency response of 66 kHz. Calibration of the pulsed doppler flowmeter was accomplished by stepwise substitution of fixed frequency sine-wave signals for the flow signals with zero frequency corresponding to zero velocity. The frequency response of the flowmeter was essentially constant in amplitude (± 3%) from zero to 66 kHz. The phase shift was linear and equivalent to a time delay of 2 msec when routed through the computer interfacing system. During data analysis, appropriate corrections were made for flowmeter phase delay relative to the pressure recording. The background instrument noise level was estimated from analysis of signals obtained during zero flow and was determined to be less than 0.1 cm/sec. There were no impedance values determined from flow harmonic components below this level in the analyzed frequency spectra.

The piezoelectric crystal was placed on the ventral surface of the aortic arch approximately midway between the innominate and left common carotid arteries and no more than 0.5 mm upstream from the proximal pressure transducer. In order to maintain consistency in technique, blood flow velocity measurements were
Figure 12. Digitized Recordings of Simultaneously Measured Aortic Arch and Abdominal Aortic Pressures in the Mouse and Rat - Control

Solid - Aortic Arch Pressure
Hatched - Abdominal Aortic Pressure

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obtained at approximately the same anatomical location in each
animal. Once optimal placement of the crystal was achieved, as
determined by the audio range output (highest clear pitch), the
crystal was glued in place with Vetbond surgical adhesive. Since
the ultrasonic beam width of the pulsed doppler crystal is
approximately 1 mm, flow velocity measurements in the aortic arch
of mice (mean external diameter 1.15 mm ± 0.02 mm) were
essentially representative of all velocities within the vessel.
In rats (mean external diameter, 2.78 mm ± 0.03 mm), the beam was
approximately concentrated on the center flow velocity determined
by signal quality and audio output.

Representative analog data recordings of aortic arch blood flow
velocity waveforms measured in mice under control and treatment
conditions are presented in figure 13. Analog measurements of
aortic arch blood flow velocity recorded in rats under all
conditions are represented in figure 14. Representative digitized
tracings of flow velocity waveforms recorded during control
conditions in rats and mice are presented in figure 15.

Upon completion of the experiment the animal was killed by
anesthetic overdose and exsanguinution, and the blood used to
conduct a post mortem flow velocity calibration. Since it was
necessary to remove the carotid artery pressure transducer in
order to perform the calibration procedure, it was not possible
to record the distance between transducers which was needed for
pulse wave velocity calculations. For this reason, flow probe
calibrations were conducted in a subset of six animals in each
Figure 13. Digitized analog recordings of flow velocity waveforms measured at the aortic arch in the mouse. Top train - control, middle train - vasoconstricted, bottom train - vasodilated.
Figure 14. Digitized analog recordings of flow velocity waveforms measured at the aortic arch in the rat. Top train - control, middle train - vasoconstricted, bottom train - vasodilated.
Figure 15. Digitized Recording of Flow Velocity Waveforms in the Mouse and Rat - Control
species and the respective mean angles were used for velocity calculations in the remaining subjects.

VASOACTIVE SUBSTANCES—ALTERING PERIPHERAL VASCULAR RESISTANCE

Baseline data were recorded after each animal had achieved and maintained a constant MAP, mean blood flow velocity, and body temperature for approximately 15 minutes. Immediately following the baseline recordings, animals received an intra-arterial injection of either Rogitine (phentolamine mesylate, an α-adrenoreceptor antagonist, PTOL) (CIBA-Geigy, Ltd. Canada., Mississauga, Ontario, Canada) or Neo-Synephrine (phenylephrine hydrochloride 1% solution, an α-adrenoreceptor agonist, PHEN) (Sterling-Winthrop, Inc., Markham, Ontario, Canada). Intra-arterial injection of vasoactive substances was chosen because peripheral venous diameters were too small to permit either introduction of intravenous cannulae or direct venipuncture in the mice. Bolus injections of Rogitine were administered at a concentration of 0.05 mg in 0.025 ml of 0.9% saline for mice, and 0.5 mg in 0.25 ml of 0.9% saline for rats. Neo-Synephrine was given as a 0.005% solution in 0.9% saline (0.025 ml volume), and as a 0.05% solution in 0.9% saline (0.25 ml volume) for mice and rats; respectively.

Pressure and flow data were collected beginning 30 seconds pre-injection and continued until each animal’s pressure returned to pre-injection status. Data trains of simultaneously recorded pressure and flow waveforms were reviewed post collection and waveforms were chosen for analysis based on the maximum response.
level which demonstrated stable pulsatile and mean pressures, consistent flow velocities and steady inter-beat intervals. Portions of data consisting of approximately 30 heart beats were isolated and pressure and flow waveform pairs were individually evaluated by Fourier analysis. The individual harmonic data points for each individual beat were averaged and mean values were taken to be representative of the amplitude of pressure and flow at each harmonic under each condition. Systemic impedance was calculated from the mean harmonic amplitudes of pressure and flow. Vasoactive challenges were randomized both within and between animals. Each animal was allowed to return to its pre-drug baseline between trials.

DATA RECORDING SYSTEM

All measurements of systolic and diastolic arterial blood pressures (aortic arch and abdominal aorta), mean and pulsatile aortic arch blood flows, and calibration values were recorded utilizing an R.C. Electronics Computerscope System (R.C. Electronics, Inc., Goleta, CA, USA). The analog outputs from the Millar micropressure transducers and pulsed doppler flow probes were interfaced with an ISC-16 analog to digital converter card and termination box (R.C. Electronics). The analog outputs were simultaneously routed to a Hewlett-Packard 3002 reel-to-reel magnetic tape recorder (Hewlett Packard Inc., Sunnyvale, CA, USA). Pressure transducers and the pulsed doppler flow assembly were checked for signal phase shifts, relative to one another, both before and after going through the data recording devices, using a signal generator. No measurable phase shifts were
observed between the pressure transducers in the range of 0.1 to 10 kHz, and 10 mV to 25 V. There was a 2 msec delay between the pressure transducers and the pulsed doppler flow signal when the signals were routed through the computer interface. Appropriate corrections for phase shift were made during the data analysis. Data sampling was conducted at a frequency of 500 Hz and the data was collected in bins ranging from 60 to 360 seconds. A series of a minimum of 50 heart beats in which pressure, flow and inter-beat intervals were stable, were converted to ascii data format and exported for analysis to Origin Analytical Software (MicroCal Software, Inc, Northampton, MA, USA).

POST MORTEM MEASUREMENTS

At the completion of the in-vivo measurements, all animals except the subset of six used for flow probe calibration were killed by anesthetic overdose. The distance between the proximal and distal cannulae was measured utilizing a Nikon hand-held surgical micrometer (resolution 0.01 mm, Nikon, Japan). Aortic tree length, defined as the distance from the innominate artery to the iliac bifurcation, was also measured with the micrometer and recorded. Measurements of aortic wall thickness and external diameter were conducted using a Nikon scope mounted optical-micrometer (resolution 0.001 mm, Nikon, Japan). All measurements were recorded in the surgical data logbook.

DETERMINATION OF AORTIC MECHANICAL PROPERTIES

Mechanical properties of the abdominal aorta, as opposed to the ascending aorta, of mice and rats were determined in situ because
isolation of the ascending aorta of mice was not technically feasible. The short length, small diameter and thin wall of the vessel, in addition to close approximation of the innominate and common carotid arteries, prevented isolation of an appropriate length for in situ measurements or removal for mounting. Measurements of the abdominal aortic mechanical properties were conducted in situ because $E_{inc}$ has been shown to be more accurate when the vessel remains tethered to the surrounding structures and longitudinal stresses are reduced (Patel and Fry, 1964). As a result, determinations of aortic compliance were conducted in the distal abdominal aorta at the completion of the experimental protocol in both species. While it is recognized that the aorta is typically less compliant at this location than it is proximally, these measurements allowed an inter-species comparison of mechanical properties and calculated values of pulse wave propagation velocities.

The abdominal aorta was occluded with a silk ligature immediately distal to the left renal artery and all peripheral vessels were ligated at the level of the aortic wall. The vessel remained as undisturbed as possible and stayed tethered to the surrounding structures both circumferentially and longitudinally. A cannula constructed of polyethylene tubing, appropriate to the vessel diameter, was introduced via the right femoral artery and advanced to the iliac bifurcation. This catheter was connected to a saline filled polyethylene line and glass bottle interfaced with a dial gauge sphygmomanometer and inflation bulb. The inflation bulb was used to apply pressure to the closed bottle.
thus forcing fluid into the abdominal aorta and creating a known
intraluminal pressure. The left femoral artery pressure
transducer was left in place to record these intra-arterial mean
pressure changes. The external diameter of the abdominal aorta
was measured with a Nikon scope mounted optical-micrometer
(resolution 0.001 mm, Nikon, Japan), at a marked 50% midpoint
between the left renal artery and the iliac artery. Diameter
measurements were recorded at pressure increments of 20 mmHg (2.7
kPa) beginning at 0 mmHg (atmospheric, open line) and continuing
to 200 mmHg (27 kPa). Since the diameter measurements were made
and recorded manually, each load-unload cycle took approximately
2 minutes. Five separate inflation trials were conducted, and the
diameter measurements were averaged for each animal to minimize
measurement error.

AORTIC TISSUE COMPOSITION

The abdominal aorta of a representative subset of animals was
infused with a 2.5% glutaraldehyde in 0.1 M sodium cacodylate/5%
sucrose fixative immediately post-mortem and harvested. Aortic
segments were fixed at 0 mmHg because technical problems
encountered in the mice prevented inflation to the species-
specific normal arterial pressure levels. After processing, the
tissue was embedded in Spurr Embedding medium for thick
sectioning (Routine Morphology Spurr Embedding Procedure, EM Lab,
Univ. of B.C., Vancouver, BC, Canada). The tissue was cut into
0.5μm sections throughout its length and stained with Toluidine
blue for routine morphological examination. Representative
segments were photographed utilizing a Zeiss Photomicroscope (C.
Zeiss, Inc. Germany).

**DATA ANALYSIS AND STATISTICS**

All data was converted to ASCII data format within the R.C. Electronics program and exported for analysis to Origin Analytical Software (MicroCal Software, Inc, Northampton, MA, USA). Descriptive statistics of cardiovascular variables were conducted with Excel Analytical Software (Microsoft, Co., Redmond, WA). A Two-Sample t-Test of baseline versus treatment group means was utilized to identify statistically significant variation ($P \leq 0.05$) in pertinent cardiovascular variables.

**BLOOD PRESSURE**

Pressure voltage signals were converted to physiologic values utilizing the calibrations recorded for each animal pre-and post-experiment. MAP was calculated from measurements of systolic and diastolic pressures according to the equation:

5) \[ \text{MAP (mmHg)} = \left\{ \frac{(\text{Systolic} - \text{Diastolic})}{3} \right\} + \text{Diastolic} \]

MAP was also determined from integration of trains of approximately 25 successive pressure pulse waveforms. The mean difference between calculated and integrated mean arterial values was found to be less than 4% under all treatment conditions for either species.

Pulse pressures were determined for both aortic arch and abdominal aortic measurements according to:
6) PP (mmHg) = Peak Systolic Pressure - Minimum Diastolic Pressure

HEART RATE DETERMINATIONS

Heart rate measurements were determined from blood pressure recordings. The inter-beat intervals were measured from the foot of the pressure pulse.

BLOOD FLOW VELOCITY

Instantaneous flow velocities were calculated utilizing the mean angle (A) obtained from post mortem calibrations conducted in the six animal subsets of each species. Analog voltages were converted to flow velocities according to the following equation:

7) \( V \text{ (mm/sec)} = \frac{(F_d \times C)}{(2 \times F_o \times \cos A)} \)

where \( V \) is blood velocity in milliliters/second, \( F_d \) is the doppler shift frequency in kHz, \( C \) is the velocity of sound in blood, \( F_o \) the transmitter frequency in kHz, and \( A \) is the angle, in degrees, between the sound beam and blood velocity vector.

STROKE VOLUME AND CARDIAC OUTPUT MEASUREMENTS

Blood flow velocity values were used in conjunction with the mean aortic arch external diameter measurement recorded at each species mean arterial pressure to calculate cardiac output (CO) as follows:

8) \( CO \text{ (cm}^3\text{/min)} = \frac{(V \text{ cm/s})(\pi)(D \text{ cm/2})^2}{60 \text{ s/min}} \)
Stroke volume (SV) was determined as:

\[ 9) \, SV \, (\text{cm}^3/\text{beat}) = \frac{\text{Cardiac Output (cm}^3/\text{min)}}{\text{Heart Rate (beats/min)}} \]

**PERIPHERAL VASCULAR RESISTANCE**

Peripheral vascular resistance (PRU) was calculated according to the equation:

\[ 10) \, \text{Peripheral Resistance PRU (dynes s}^{-1} \, \text{cm}^{-3}) = \left[ \frac{(\text{MAP dynes} / 10)(980 \, \text{cm} \, / \, \text{s}^2)}{(\text{CO} \, \text{cm}^3 \, / \, \text{s})} \right] \]

**PULSE WAVE VELOCITY MEASUREMENTS**

Pressure pulse wave velocity was determined from blood pressure and distance measurements. The micropressure transducer tip-to-tip distance was used, in conjunction with simultaneously recorded proximal and distal pressure pulses, to calculate the pulse wave velocity according to the equation:

\[ 11) \, C \, (\text{m/s}) = \frac{\Delta z}{\Delta t} \]

where \( \Delta z \) is distance in meters and \( \Delta t \) is the time delay in seconds. The time-derivative of the pressure pulse wave was calculated and the transit time of the peak of the derivative was then determined and taken to be characteristic of the apparent wave velocity. Aortic tree lengths and the pulse wave velocity values were used to determine the pressure wavelength of the fundamental frequency according to the relationship:
12) \( \lambda = \frac{C}{f_o} \)

where \( C \) is the apparent pulse wave velocity and \( f_o \) is the heart rate in beats/s. The aortic length and wavelength values were then used to determine the \( L : \lambda \) ratio and pulse transit time as a percentage of the cardiac cycle. Pulse transit time was calculated as follows:

13) \( \%CC = \left[ \frac{L}{C} / \left( \frac{1 \text{ sec}}{f_o} \right) \right] \times 100 \)

INPUT IMPEDANCE DETERMINATIONS

Simultaneous measurements of pressure and flow waveforms were subjected to Fourier Series analysis (Origin Analytical Tools, MicroCal Software, Inc., Northampton, MA, USA) to determine the energy distribution characteristics. A single sequence consisted of a 5 - 10 second window of events (approximately 40-60 consecutive heartbeats). Each pressure/flow waveform pair was analyzed individually. The impedance moduli of each individual harmonic was then averaged for the entire sequence. The averaged spectrum was taken to be representative of the systemic vascular impedance of each animal. The impedance values were calculated according to the following equations:

14a) \( Z_n = \frac{P_n}{Q_n} \)

14b) \( \theta_n = (\beta_n - \alpha_n) \)

where A) \( P_n \) is the peak pressure amplitude and \( Q_n \) is peak flow amplitude at the \( n \)th harmonic, and B) \( \theta \) is the phase calculated from \( \beta_n \), and \( \alpha_n \), the phase angles at each harmonic of the flow and pressure pulses; respectively.
WINDKESSEL PREDICTION - 3 ELEMENT MODEL

A 3-component Windkessel Model (Burkhoff et al., 1988) was used to describe the impedance moduli and phase, as well as the characteristic impedance, which would be expected if the arterial system functioned in the absence of significant wave reflections. Mean values of heart rate, diastolic time decay of the pressure pulse, and pulse wave velocity were utilized to perform the calculations for the control and treatment groups. Impedance calculations were performed according to the following equations:

\[ Z_{\text{in}}(\omega) = R_c + [R_a \div \{1 + (j\omega C_a R_a)\}] \]

where \( Z_{\text{in}} \) is the impedance modulus at each value of angular frequency \( \omega \) (\( \omega = 2\pi f \), when \( f \) is heart rate in Hz), \( R_c \) is the characteristic impedance determined from the arithmetic mean of \( Z \) amplitudes above (and including) the 1st harmonic, \( R_a \) is the difference between the zero harmonic amplitude of impedance and \( R_c \), \( \omega \) is equal to \( 2\pi f \) where \( f \) is equal to the heart rate, \( j \) is equal to an imaginary number equal to \( \sqrt{-1} \), and \( C_a \) is equivalent to \( T / R_a \), where \( T \) is equal to the diastolic time decay of the pressure pulse. The phase of impedance was calculated according to the equation:

\[ \beta = (\omega / C) \]

where \( \beta \) is equal to the phase angle, and \( C \) is equal to the pulse wave velocity in m/s.
DETERMINATION OF PRESSURE PULSE AMPLIFICATION AND DISTORTION

Individual pressure waveform pairs were compared by Fourier Series analysis to quantify potential pulse amplification and distortion effects between the heart and the periphery. These comparisons elucidate alterations in amplitude at each harmonic frequency thus quantifying the potential interference of reflected waves. Individual pulse waveforms were also visually inspected by direct comparison of each pair of digitized analog waveforms.

ARTERIAL BIOMECHANICAL PROPERTIES

Arterial wall stress, circumferential wall strain, and the incremental elastic modulus of the abdominal aorta were calculated, assuming a constant wall volume and linear deformation with applied stress, according to the following equations:

17) Circumferential Stress $\sigma_c$ (kPa) = $(P)(r) / (h)$

where $P$ is pressure (kPa), $r$ is internal radius (m), and $h$ is wall thickness (m).

18) Circumferential Wall Strain $\epsilon$, (at midwall radius)

$$\epsilon = \Delta \tilde{R} / R_0$$

$$\tilde{R} = (R + r) / 2$$

where $\tilde{R}$ is the external radius (m) and $R_0$ (m) is the unpressurized midwall radius.
19) Incremental Elastic Modulus $E_{inc}$ (at constant length)

$$E_{inc} \ (kPa) = (1 - \mu^2) \ (1 + \varepsilon) \ \left( \Delta\sigma_c / \Delta\varepsilon \right)$$

where $\mu$ is Poisson's ratio which is assumed to be 0.5 for arterial vessel walls, $\Delta\sigma \ (kPa)$ is the change in circumferential wall stress and $\Delta\varepsilon$ is the change in circumferential wall strain at each pressure increment (Bergel, 1961).

The lamellae-thickness ratio for the abdominal aortic segments was calculated from the total wall thickness and number of lamellae according to the equation:

$$20) \ L_m \cdot h = \text{(Total Number of Lamellae)} \div \text{(wall thickness)}$$

PREDICTED VALUES OF PULSE WAVE VELOCITY AND TRANSIT TIME

In animals for which pressure-radii loop measurements were obtained, the predicted velocity of pressure pulse wave propagation was calculated according to the equation:

$$21) \ C_0 \ (m/s) = \left[ (E_{inc}) \ (h) / (2\alpha) \ (R_{in}) \right]$$

where $E_{inc}$ is the incremental elastic modulus (kPa), $h$ is the aortic wall thickness (m), $\alpha$ is the viscosity of blood (kPa m$^{-2}$ s$^{-1}$), and $R_{in}$ is the inside vessel radius (m). The predicted pulse transit time as a percentage of the cardiac cycle was also calculated from the following equation:

$$22) \ %CC_{\text{pred}} = \left[ \left( L / C_0 \right) / \left( 1 \ \text{sec} / f_0 \right) \right] \times 100$$
where $C_0$ is the predicted velocity of pressure pulse wave propagation ($\text{m s}^{-1}$), $L$ is aortic length (m) and $f_0$ is the fundamental frequency of contraction ($\text{s}^{-1}$).

**ALLOMETRIC ESTIMATIONS OF CARDIOVASCULAR AND ANATOMICAL VARIABLES**

Estimations of heart rate, stroke volume, cardiac output, aortic length and total peripheral resistance were calculated from allometric equations (Holt et al., 1968; Schmidt-Nielsen, 1984) as follows:

23) Heart Rate (beats/sec) = $3.93 M^{-0.25}$

24) Stroke Volume (mls/beat) = $0.66 M^{1.05}$

25) Cardiac Output (mls/min) = $187 M^{0.81}$

26) Aortic Length (m) = $M^{0.33}$

A mean body weight of 0.0345 kg was used in all calculations for mice (mean control group value) and a mean body weight of 0.579 kg (mean control group value) was used for rats.
CHAPTER III.
RESULTS

SECTION 1: MICE

ANATOMICAL DETERMINATIONS
Mean body weights of control (C), vasodilation (PTOL) and vasoconstriction (PHEN) treatment groups were 0.0347 ± 0.002 (n=14), 0.0346 ± 0.002 (n=8), and 0.0315 ± 0.003 (n=8) kgs; respectively. Mean aortic length for C was 0.0394 ± 0.003 m, for PTOL 0.0402 ± 0.002 m, and for the PHEN group 0.0394 ± 0.003 m. Body weights and aortic lengths of animals from the PTOL and PHEN groups were included in the control mean values. There were no statistical differences in mean mass or aortic lengths between groups. Mean aortic arch external diameter at zero pressure was 1.16 ± 0.01 mm and mean wall thickness was 0.01 ± 0.002 mm.

CARDIOVASCULAR VARIABLES
Group mean values of pulse wave velocity (C, equation 11), pressure pulse wavelength (λ, equation 12), aortic length/wavelength ratio (L/λ), pressure pulse transit time as percentage of cardiac cycle (%CC, equation 13), mean arterial pressure (MAP, equation 5), peak systolic-minimum diastolic pressure differential at the aortic arch (PParch, equation 6) and abdominal aorta (PPaorta), heart rate (HR), cardiac output (CO, equation 8), stroke volume (SV, equation 9), blood flow velocity (V, equation 7), and peripheral vascular resistance (Rs, equation 10) are presented in Table 1. Cardiovascular variables were
Table 1 Cardiovascular Variables in Mice. Mean Values for Control, Vasodilation (PTOL) and Vasoconstriction (PHEN) Treatment Groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>PTOL</th>
<th>PHEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>PWV (m/s)</td>
<td>8508</td>
<td>3507</td>
<td>827</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>89.4</td>
<td>94.7</td>
<td>96</td>
</tr>
<tr>
<td>%CC</td>
<td>67</td>
<td>52</td>
<td>51</td>
</tr>
<tr>
<td>HR (beats/sec)</td>
<td>274</td>
<td>276</td>
<td>273</td>
</tr>
<tr>
<td>CO (mls/sec/kg)</td>
<td>2.9</td>
<td>3.0</td>
<td>3.1</td>
</tr>
<tr>
<td>SV (mls/beat/kg)</td>
<td>47</td>
<td>48</td>
<td>47</td>
</tr>
<tr>
<td>Rs (dynes-s / cm^5 /kg)</td>
<td>2.5</td>
<td>2.6</td>
<td>2.7</td>
</tr>
<tr>
<td>Flow Velocity (cm / sec)</td>
<td>5.3</td>
<td>5.4</td>
<td>5.5</td>
</tr>
</tbody>
</table>

* = Statistically different than Control Value at P < 0.05
also standardized to body mass and are expressed on a "per kilogram" basis. Statistically significant differences (T \leq t, Unpaired T-test Assuming Unequal Variances) were found for mean values of C (P = 0.050), MAP (P = 0.017), Rs (P = 0.047), HR (P = 0.001), CO (P = 0.032), and SV (P = 0.008) in the PHEN treatment group as compared with control. There were no statistically significant differences in the remaining variables between PHEN and control groups. There were no statistically significant differences at P \leq 0.05 for any variable between the PTOL and control group mean values.

In each group, cardiovascular variables were calculated as the percentage of change from each individual's control values. Mean change in these variables, expressed as percentage of control, are presented in Table 2 and are represented graphically in figures 16 and 17. In the PHEN group, pulse wave velocity was 12.8 ± 2% greater than control values. Mean peripheral vascular resistance increased by 126 ± 35.4%, heart rate was elevated by 26.4 ± 19.9%, and mean arterial pressure rose 45.6 ± 5.4% over control levels. There was a 19.7 ± 23.4% decrease in stroke volume, and no significant change in cardiac output at 1.0 ± 28.4%. In the PTOL trials, both mean pulse wave velocity and heart rate decreased insignificantly, to -3.2 ± 2.2% and -1.6 ± 5.7% of control values; respectively. Mean stroke volume increased by 29.9 ± 39.9% and cardiac output increased by 29.3 ± 37.2%. Mean peripheral resistance fell by 40.0 ± 17.2%, however the mean arterial pressure was largely unchanged at 6.5 ± 0.2% above control values.
Table 2  Alterations in Cardiovascular Variables in Mice expressed as Percentage of Control Values.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>PTOL</th>
<th>Stand.Dev.</th>
<th>N</th>
<th>PHEN</th>
<th>Stand.Dev.</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulse Wave Velocity</td>
<td>0</td>
<td>0</td>
<td>-3.2</td>
<td>2.2</td>
<td>6</td>
<td>12.8</td>
<td>2</td>
</tr>
<tr>
<td>Heart Rate</td>
<td>0</td>
<td>0</td>
<td>-1.6</td>
<td>5.7</td>
<td>8</td>
<td>26.4</td>
<td>19.9</td>
</tr>
<tr>
<td>Stroke Volume</td>
<td>0</td>
<td>29.9</td>
<td>39.9</td>
<td>37.2</td>
<td>8</td>
<td>-19.7</td>
<td>23.4</td>
</tr>
<tr>
<td>Cadiac Output</td>
<td>0</td>
<td>29.3</td>
<td>37.2</td>
<td>39.9</td>
<td>8</td>
<td>1</td>
<td>28.4</td>
</tr>
<tr>
<td>Peripheral Resistance</td>
<td>0</td>
<td>-40</td>
<td>17.2</td>
<td>126</td>
<td>8</td>
<td>35.4</td>
<td>7</td>
</tr>
<tr>
<td>Mean Arterial Pressure</td>
<td>0</td>
<td>6.5</td>
<td>0.2</td>
<td>45.6</td>
<td>5.4</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

Figure 16: Changes in Heart Rate, Stroke Volume, and Cardiac Output in Mice, expressed as Percent of Control.
Figure 17. Changes in Pulse Wave Velocity, Peripheral Vascular Resistance, and Mean Arterial Pressure in Mice, expressed as Percent of Control.
SYSTEMIC VASCULAR IMPEDANCE

Systemic vascular impedance values were calculated from analysis of individual pressure/flow waveform pairs. These pairs were isolated from a sequence of approximately 50 consecutive heartbeats recorded during a period of consistent inter-beat intervals. The impedance moduli of each individual harmonic was then averaged for the entire sequence, and the mean spectrum was taken to be typical of the vascular impedance of each animal. Representative digitized analog recordings of pressure and flow waveforms obtained under control conditions are presented in figure 18. Simultaneously recorded waveforms obtained during vasoactive drug administration are presented in figure 19.

All impedance values (equations 14a, 14b) are expressed in dyn sec/cm$^3$. Control values of impedance modulus and phase are presented in figure 20. Systemic vascular impedance values were also normalized to each animal's peripheral resistance and are represented in figure 21. In all animals (n=11), there was a rapid decrease in impedance amplitude from the first harmonic, reaching a minimum value by the second harmonic. The initial decline was followed by a continual, gradual fall in impedance amplitude with increasing frequency. In some animals, there were very small fluctuations between the second and fifth harmonics but there were no apparent minima or maxima. Phase of impedance remained below zero throughout the harmonic sequence analyzed. These results indicate an absence of discrete wave reflection effects.
Figure 18: Digital analog recordings of simultaneously measured arterial and venous pressure (mmHg) and flow velocity (cm/sec) waveforms in the mouse.
Figure 19. Digitized analog recordings of simultaneously measured aortic arch pressure (heavy line) and flow velocity (light line) waveforms in the mouse. Top train — vasoconstricted, bottom train — vasodilated.
Figure 20. Systemic Vascular Impedance Modulus and Phase of Individual Mice - Control Values.
Figure 21. Systemic Vascular Impedance Modulus (Normalized to Peripheral Resistance) and Phase of Individual Mice - Control Values
Vasoconstriction (PHEN) impedance and phase spectra are represented in figure 22. Normalized impedance moduli are presented in figure 23. In the PHEN treatment group (n=7), the magnitude of impedance at the fundamental frequency was not significantly changed from control values for all animals. In general, there was a more gradual decline in amplitude of impedance with increasing frequency following the first harmonic. While there were no grossly apparent minima or maxima for any animal, two animals showed a small increase in amplitude at the third harmonic followed by a steady decline with increasing frequency. Two additional animals displayed a small but steady increase in impedance from the fourth through the seventh harmonics. Although phase remained below zero throughout the frequency spectrum analyzed, there were more fluctuations in the lower frequencies. The small fluctuations in the higher harmonics, and the less negative phase, indicate that there may be an increase in peripheral reflections, however, the presence of discrete effects on systemic impedance are not apparent.

Vasodilation (PTOL) impedance moduli and phase are graphically presented in figure 24. Impedance moduli, normalized to peripheral resistance values for each mouse are presented in figure 25. In the PTOL treatment group (n=7), all animals experienced an increase in impedance amplitude at the fundamental harmonic as compared with control values. In four animals, impedance moduli decreased in amplitude with increasing frequency while the remaining three mice settled at a minimal value by the second harmonic and remained at that value through the remaining
Figure 22. Systemic Vascular Impedance Modulus and Phase of Individual Mice - Vasoconstriction Induced by 0.005% Phenylephrine
Figure 23. Systemic Vascular Impedance Modulus (Normalized to Peripheral Resistance) and Phase of Individual Mice - Vasoconstriction induced by 0.005% Phenylephrine
Figure 24. Systemic Vascular Impedance Modulus and Phase of Individual Mice - Vasodilation Induced by 0.05 mg Phentolamine

The graph shows the systemic vascular impedance modulus and phase for individual mice after vasodilation induced by 0.05 mg Phentolamine. The impedance modulus is measured in dynes s cm⁻¹, and the phase is measured in radians. The data is presented for various harmonics, and each line corresponds to a different mouse (e.g., m302, m309, m1007, etc.).
Figure 25. Systemic Vascular Impedance Modulus (Normalized to Peripheral Resistance) and Phase of Individual Mice - Vasodilation Induced by 0.05 mg Phentolamine
harmonics. Phase values remained below zero, tended to be more negative than control values and displayed less fluctuation than the control values.

The mean harmonic impedance amplitudes and phases for all mice were averaged within the control, PHEN, and PTOL groups to produce a group mean impedance spectrum. These values are presented in figure 26. Statistical analysis of the impedance amplitude and phase were conducted for each harmonic utilizing a Two-sample t-Test assuming equal variance (P ≤ 0.05). There were no significant differences in amplitude or phase between the control and PHEN groups at any harmonic. Impedance amplitude was greater at the second and third harmonics in the PTOL group than in the control (P = 0.041 and P = 0.049; respectively). The phase of impedance at the second harmonic in the PTOL group was statistically significantly less than that of the control (P = 0.039). The remaining values of impedance and phase were not different statistically.

PRESSURE PULSE AMPLIFICATION AND DISTORTION
Simultaneous recordings of aortic arch and abdominal aortic pressure pulses were obtained and compared to elucidate differences in peak systolic pressure, rise time, pulsatile pressure and diastolic decline intervals. Representative analog recordings of central and peripheral waveforms under control, vasoconstriction and vasodilation conditions are presented in figure 27.
Figure 26: Mean Systemic Impedance in Mice - Control vs. PHEN and PTOL (Group Mean +/- SE)

**IMPEDANCE (dyn - s / m²)**

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

**HARMONIC**

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

**PHASE (radians)**

-3.0 -2.5 -2.0 -1.5 -1.0 -0.5 -0.0 0.0 0.5 1.0 1.5 2.0 2.5 3.0

- = Statistically Different than Control Impedance Value

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Figure 27. Digitized analog recordings of simultaneously measured aortic arch (heavy line) and abdominal aortic (light line) pressures in the mouse. Top: control; middle: vasoconstriction; bottom: vasodilation.
A representative digitized tracing of simultaneously recorded individual pressure pulses and the corresponding Fourier analysis for a control animal is presented in figure 28. Examination of paired pressure pulses showed little or no increase in peak systolic pressure in the abdominal aorta as compared with the aortic arch recording (within the resolution of the micromanometers, 1 mmHg per 100 mmHg) in any animal. In all animals, diastolic pressures were lower in the abdominal aorta than in the aortic arch by several mmHg. The group mean aortic arch pulse pressure was slightly greater than the mean abdominal aortic pulse pressure (mean 29.6 ± 15.5 mmHg versus 27.4 ± 6.2 mmHg; respectively), but these values were not statistically different (at P ≤ 0.05). Centrally, there was no visible incisura in any pressure tracing, and the amplitude of the dicrotic wave varied from animal to animal. In all animals, the dicrotic wave was not visible in the peripheral pressure recordings under any condition. Harmonic analysis of simultaneously recorded pressure pulses illustrated a general pattern of decreasing amplitude with increasing frequency in both waveforms. Within the error of margin of the micropressure transducers, the amplitudes of the central and peripheral pulses were not greatly different at any harmonic frequency.

In the PHEN group, peripheral vasoconstriction often resulted in occlusion of the abdominal aortic cannula thus limiting the number of subjects in which pulsatile pressures could be recorded. In the three subjects measured there was a very small but apparent peripheral pulse amplification. The mean pulsatile
Figure 28. Simultaneously Recorded Aortic Arch and Abdominal Aortic Pressures in a Mouse - Control
Top Graph - Digitized Recording, Bottom Graph - Fourier Analysis of Paired Pressure Pulse

Mean Measurement Error per 100 mmHg = 1%
arch pressure of the subgroup was 25.3 ± 1.12 mmHg while the
PPaorta was 27.2 ± 0.99 mmHg. In each animal, the increase in
peripheral peak pressure was accompanied by a more rapid systolic
rise as compared with the central pulse. The diastolic decline
pattern and waveform of each pulse was similar but there was no
apparent dicrotic wave in any peripheral pressure recording.
Harmonic analysis generally showed a greater amplitude at the
first harmonic frequency in the PPaorta than in the PParch
(averaging 4 mmHg) and a slightly greater amplitude through the
fourth harmonic. The relative amplitudes of the harmonic
components of each pulse changed synchronously indicating that
there was little difference in wave shape despite a small
increase in peak pressure peripherally. A representative paired
pressure pulse tracing and the corresponding Fourier analysis are
presented in figure 29.

A representative paired PParch and PPaorta tracing, as well as
the accompanying Fourier analysis, from the PTOL vasodilation
group is presented in figure 30. While mean pulsatile pressures
measured at the aortic arch (29.3 mmHg ± 15.8) were not
significantly different from control values mean peripheral
pulsatile pressures declined to 23.0 ± 7.1 mmHg. Although there
was a fall in peak systolic pressures peripherally, there was
little difference in wave shape as compared with the central
pulse. The Fourier analyses demonstrated a lower PPaorta
amplitude at the first harmonic than the PParch amplitude and no
detectable differences in the remaining frequency components of
the two pulses.
Figure 29. Simultaneously Recorded Aortic Arch and Abdominal Aortic Pressures in a Mouse-Vasoconstriction Induced by 0.005% Phenylephrine
Top Graph - Digitized Recording, Bottom Graph - Fourier Analysis of Paired Pressure Pulse

Mean Measurement Error per 100 mmHg < 1%
Figure 30. Simultaneously Recorded Aortic Arch and Abdominal Aortic Pressures in a Mouse - Vasodilation Induced by 0.05 mg Phentolamine
Top Graph - Digitized Recording, Bottom Graph - Fourier analysis of Paired Pressure Pulse

Mean Measurement Error per 100 mmHg = 1%
FLOW VELOCITY WAVEFORMS

A pair of simultaneously recorded digitized aortic arch pressure and flow waveforms are presented in figure 31. This flow waveform is representative of recordings obtained in both control and treatment group animals. The amplitude of the flow incisura varied between animals and the duration of ejection changed with heart rate. Recordings obtained in the treatment groups varied slightly in contour as was expected due to alterations in ejection patterns and afterload. Although mean flow velocity increased in the PHEN (45.2 ± 13.1 cm/sec) and decreased in the PTOL (23.9 ± 9.1 cm/sec) treatment groups as compared with control (30.8 ± 12.6 cm/sec), these alterations were not statistically significant at P ≤ 0.05.

WINDKESSEL PREDICTIONS

Predicted values, as well as mean values of characteristic impedance, systemic input impedance moduli and phase for control and treatment groups (equations 15 and 16) are presented in table 3. The characteristic impedance values were calculated from the arithmetic mean of the harmonic components above and including the first harmonic. The values of systemic impedance at each harmonic frequency were normalized to characteristic impedance for both the mathematical model and for the "mean" mouse and are presented graphically in figure 32.

Under all treatment conditions, the 3-Component model showed a rapid decline from the characteristic impedance amplitude at zero to a low value at the first harmonic followed by a more gradual
Figure 31. Simultaneously Recorded Aortic Arch Pressure and Flow Velocity Waveforms in a Mouse

Pressure (mmHg)

Flow Velocity

Time (sec)

Flow Velocity (cm/sec)
Table 3  Predicted 3-Component Windkessel versus Mean Values of Systemic Vascular Impedance in Mice. Control, Vasodilation, and Vasoconstriction Treatment Groups.

<table>
<thead>
<tr>
<th>Harmonic</th>
<th>Control Impedance</th>
<th>Control Phase</th>
<th>Vasodilation Impedance</th>
<th>Vasodilation Phase</th>
<th>Vasoconstriction Impedance</th>
<th>Vasoconstriction Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0.1784</td>
<td>0.1542</td>
<td>0.1951</td>
<td>-0.5074</td>
<td>0.0945</td>
<td>-0.2988</td>
</tr>
<tr>
<td>2</td>
<td>0.1182</td>
<td>0.1024</td>
<td>0.1548</td>
<td>-0.7109</td>
<td>0.0665</td>
<td>-0.5976</td>
</tr>
<tr>
<td>3</td>
<td>0.0998</td>
<td>0.1054</td>
<td>0.1405</td>
<td>-0.6691</td>
<td>0.1163</td>
<td>-0.8964</td>
</tr>
<tr>
<td>4</td>
<td>0.0878</td>
<td>0.1115</td>
<td>0.1331</td>
<td>-0.5875</td>
<td>0.3188</td>
<td>-1.1952</td>
</tr>
<tr>
<td>5</td>
<td>0.0671</td>
<td>0.1168</td>
<td>0.1286</td>
<td>-0.6126</td>
<td>0.1424</td>
<td>-1.4941</td>
</tr>
<tr>
<td>0</td>
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<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0.2411</td>
<td>0.0685</td>
<td>0.2009</td>
<td>-0.5204</td>
<td>0.1592</td>
<td>-0.3331</td>
</tr>
<tr>
<td>2</td>
<td>0.1451</td>
<td>0.0377</td>
<td>0.1711</td>
<td>-0.7438</td>
<td>0.1883</td>
<td>-0.6761</td>
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<tr>
<td>3</td>
<td>0.1031</td>
<td>0.02</td>
<td>0.1553</td>
<td>-0.4756</td>
<td>0.1486</td>
<td>-1.3323</td>
</tr>
<tr>
<td>4</td>
<td>0.0939</td>
<td>0.0203</td>
<td>0.1521</td>
<td>-0.7267</td>
<td>0.1702</td>
<td>-1.5654</td>
</tr>
</tbody>
</table>
Figure 32. Mean Systemic Vascular Impedance in Mice (Normalized to Characteristic Impedance)
Average Measured Values vs. 3 - Component Windkessel Model Predictions for Control, PHEN and PTOL Groups
fall in amplitude through the fifth harmonic component. There were no predicted minima or maxima and no fluctuations in amplitude. The phase declined gradually from zero radians at zero to reach a maximum negative value at the fifth harmonic.

In the empirical mouse, calculated from mean values measured under control conditions, the impedance amplitude was below predicted values at all harmonics and lacked any maxima or minima. Although phase remained negative throughout the frequency spectrum, a minimum value was reached at the second harmonic followed by a small rise through the fifth harmonic.

In the PHEN group, the average impedance amplitude was greater than the predicted value at the first harmonic but fell below predicted values at the second harmonic. Amplitude remained less than predicted through the frequency spectrum and again, there were no maxima or minima. Phase remained negative throughout the frequency spectrum but gradually rose from a minimum value at the first harmonic to the fourth, declining slightly at the fifth harmonic.

In the PTOL group, impedance amplitude was slightly greater than the predicted value at the first harmonic but declined rapidly through the remaining harmonics with amplitudes below predicted values. Again, there were no apparent maxima or minima at any frequency. Phase fell from zero radians to a minimum value at the first harmonic, rising gradually through the fifth. There were no fluctuations in phase.
Figure 33 illustrates the circumferential stress-strain behavior (equations 17 and 18) and incremental elastic moduli ($E_{inc}$) of the abdominal aorta (equation 19). At a mean physiologic pressure of 60 mmHg (~8.2 kPa) the elastic modulus was 258 kPa. The stress-strain curve for the abdominal aorta illustrates a gradual increase in stiffness with increasing strain. The abrupt increase in stiffness normally observed at the upper physiological pressure range in mammalian vessels is not as readily apparent in the mouse abdominal aorta. At stresses of about 11 kPa (80 mmHg) the slope begins to increase more sharply but does not demonstrate a rapid incline until stress exceeds 16 kPa (120 mmHg). These results indicate a greater elasticity than most mammalian abdominal aortic vessels and are reflective of the lower pulse wave velocities measured in mice.

The predicted velocity of pressure pulse wave propagation ($C_o$, equation 21) and predicted pressure pulse transit time expressed as percentage of cardiac cycle ($%CC_{pred}$, equation 22) were calculated from the biomechanical measurements. These values, as well as the measured values, are presented in table 4. The measured $C$ (mean = 2.599 ± 0.07 m/s) for all animals was 10% less than the predicted values (mean = 2.880 ± 0.02 m/s). The average measured pulse transit times occupied 10.1 ± 0.67% of the cardiac cycle while the mean predicted value was 9.12 ± 0.71%. The $C_o$ calculated from biomechanical data is in close agreement with the measured values and reflects the extreme elasticity of the central vessels.
Figure 33. Circumferential Stress-Strain Relationship (+/- sd), and Incremental Elastic Modulus as a Function of Distending Pressure (+/- sd), in the Abdominal Aorta of Mice.
Table 4. Pressure Wave Velocity and Transit Time Calculated from Mechanical Properties versus Measured Values in Mice

<table>
<thead>
<tr>
<th>Animal Number</th>
<th>Measured C (m/s)</th>
<th>Predicted C (m/s)</th>
<th>Measured %CC</th>
<th>Predicted %CC</th>
</tr>
</thead>
<tbody>
<tr>
<td>302</td>
<td>2.63</td>
<td>2.9</td>
<td>10.9</td>
<td>9.9</td>
</tr>
<tr>
<td>309</td>
<td>2.65</td>
<td>2.86</td>
<td>9.8</td>
<td>9.1</td>
</tr>
<tr>
<td>315</td>
<td>2.52</td>
<td>2.88</td>
<td>9.7</td>
<td>8.4</td>
</tr>
</tbody>
</table>

| Mean          | 2.6              | 2.9               | 10.1         | 9.1           |
| Standard Deviation | 0.07           | 0.02              | 0.7          | 0.7           |
AORTIC TISSUE COMPOSITION

Figure 34 illustrates the anatomical composition of the abdominal aortic wall. The top photograph depicts a complete cross-sectional segment, with a closeup presented beneath. The internal elastic lamella is clearly visible at the interface of the tunica intima and luminal space, with the external surface demarcated by the adventitia. The aortic wall is demarcated by three visible concentric elastic bands within the tunica media. The abdominal aortic wall is relatively thin, and has a lamellae/thickness ratio of 0.33 (equation 22), lower than that of the rat (0.41) which is more typical of mammalian central arteries (Wolinsky, 1964). These histological results are consistent with the findings of the biomechanical stress testing.

ALLOMETRIC PREDICTIONS OF CARDIOVASCULAR VARIABLES

Allometrically predicted and mean measured values of L (equation 26), HR (equation 23), SV (equation 24), and CO (equation 25) of control animals are presented in table 5. The cardiovascular variables are also represented graphically in figure 35. All predicted values were calculated from the mean body mass of the control group (0.0345 kg). Mean L was greater than the predicted value by 31%. SV was greater than the predicted by 24%. Mean HR was 22% less than the predicted values while mean CO was only 1% greater. Although mean arterial pressure (MAP) has not been demonstrated to scale allometrically, these animals had a MAP approximately 30% lower than other mammalian species.
Figure 34. The abdominal aorta of a mouse. Cross-sections fixed at 0 distending pressure. Top photograph - Whole aorta, 440x magnification. Bottom photograph - Wall segment, 880x magnification.
Table 5. Allometrically Predicted versus Measured Cardiovascular Variables in the Mouse.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Predicted Value</th>
<th>Measured Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aortic Length (meters)</td>
<td>0.0302</td>
<td>0.0394</td>
</tr>
<tr>
<td>Heart Rate (beats/second)</td>
<td>9.12</td>
<td>7.45</td>
</tr>
<tr>
<td>Pulse Wave Velocity</td>
<td>4.4</td>
<td>2.73</td>
</tr>
<tr>
<td>Length/Lambda</td>
<td>0.06</td>
<td>0.11</td>
</tr>
<tr>
<td>Stroke Volume (mls/beat/kg)</td>
<td>0.558</td>
<td>0.689</td>
</tr>
<tr>
<td>Cardiac Output (L/min/kg)</td>
<td>0.305</td>
<td>0.308</td>
</tr>
</tbody>
</table>
Figure 35. Allometrically Predicted versus Measured Cardiovascular Variables in the Mouse.

- Mean Measured Heart Rate (bpm)
- Predicted Heart Rate (a=3.93, b=-0.25)
- Measured Stroke Volume (mL/bpm/kg)
- Predicted Stroke Volume (a=0.66, b=1.05)
- Measured Cardiac Output (L/min/kg)
- Predicted Cardiac Output (a=187, b=0.81)

(Allometric values from Holt et al., 1968)
SECTION 2: RATS

ANATOMICAL DETERMINATIONS
Mean body weights of control (C), vasodilation (PTOL), and vasoconstriction (PHEN) treatment groups were 0.573 ± 0.048, 0.582 ± 0.042, and 0.577 ± 0.048 kgs; respectively. Aortic length mean values for C, PTOL, and PHEN groups were 0.0995 ± 0.0082, 0.1037 ± 0.0085, and 0.0985 ± 0.0043 m; respectively. All animals in the vasoactive treatment groups were included in mean control values. There were no statistically significant differences at a level of P ≤ 0.05 in mean body mass or aortic lengths between groups. Mean aortic arch external diameter at zero pressure was 1.25 ± 0.01 mm, and mean wall thickness was 0.037 ± 0.0023 mm.

CARDIOVASCULAR VARIABLES
Group mean values of C, λ, L/λ, %CC, MAP, PParach, PPaorta, HR, CO, SV, V, and Rs, are presented in Table 6. When appropriate, cardiovascular variables were standardized to body mass and are expressed on a "per kilogram" basis. Statistically significant differences (T ≤ t, Unpaired T-test Assuming Unequal Variances) were found at P ≤ 0.05 for group values of C (P = 0.044), λ (P = 0.047), L/λ (P = 0.018), Rs (P = 0.017) and MAP (P = 0.003) in the PHEN treatment group as compared with control. There were no statistically significant differences in the remaining variables in this group. Statistically significant differences were found between control and mean values of λ (P = 0.01), L/λ (P = 0.011), Rs (P = 0.039), MAP (P = 0.19 x 10^{-6}), SV (P = 0.05) and PPaorta (P = 0.003) in the PTOL group. The remaining variables were not
Table 6. Cardiovascular Variables in Rats. Mean Values for Control, Vasodilation (PTOL), and Vasoconstriction (PHEN) Treatment Groups.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>PWV (meters)</td>
<td>7.758±6</td>
<td>0.699±0.13</td>
<td>9</td>
<td>4.10±0.22</td>
<td>0.75±0.14</td>
<td>9</td>
<td>6.92±0.16</td>
<td>0.70±0.12</td>
<td>6</td>
</tr>
<tr>
<td>% CC</td>
<td>92.7±2.4</td>
<td>93.9±2.1</td>
<td>11</td>
<td>32.6±1.3</td>
<td>40.6±2.2</td>
<td>6</td>
<td>8.7±0.2</td>
<td>11</td>
<td>32.6±1.3</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>45.9±4</td>
<td>4.8±0.4</td>
<td>11</td>
<td>39.5±3.2</td>
<td>32.6±1.3</td>
<td>6</td>
<td>13.0±1.1</td>
<td>11</td>
<td>39.5±3.2</td>
</tr>
<tr>
<td>CO (mls/sec/kg)</td>
<td>25.8±1.4</td>
<td>25.8±1.4</td>
<td>6</td>
<td>5.4±0.7</td>
<td>5.3±0.3</td>
<td>6</td>
<td>4.8±0.8</td>
<td>11</td>
<td>5.4±0.7</td>
</tr>
<tr>
<td>SV (mls/beat/kg)</td>
<td>18.3±0.7</td>
<td>18.3±0.7</td>
<td>11</td>
<td>5.1±0.4</td>
<td>5.6±0.5</td>
<td>6</td>
<td>4.8±0.8</td>
<td>11</td>
<td>5.1±0.4</td>
</tr>
<tr>
<td>Flow Velocity (cm/sec)</td>
<td>1.3±0.2</td>
<td>1.3±0.2</td>
<td>11</td>
<td>0.4±0.1</td>
<td>0.4±0.2</td>
<td>6</td>
<td>0.5±0.1</td>
<td>11</td>
<td>0.4±0.1</td>
</tr>
<tr>
<td>Rs (dynes-s/cm³/kg)</td>
<td>0.12±0.04</td>
<td>0.12±0.04</td>
<td>6</td>
<td>0.2±0.06</td>
<td>0.2±0.06</td>
<td>6</td>
<td>0.1±0.04</td>
<td>11</td>
<td>0.2±0.06</td>
</tr>
</tbody>
</table>

* = Statistically different than Control at P < 0.05
Mean percent change from control values for each group are presented in table 7 and are represented graphically in figures 36 and 37. In the PHEN group, C increased by 65%, $R_s$ increased by 72%, HR was elevated by 15.2%, and MAP rose by 40.6% over control levels. There was an 18.4% decrease in SV, and a 8.6% decrease in cardiac output below control values. In the PTOL trials, C decreased by 12.8%, SV was reduced by 29.3%, and CO fell by 24.7%. HR increased by 7.5% over the mean control value. $R_s$ fell by 36% and the MAP was decreased by 90.2%.

SYSTEMIC VASCULAR IMPEDANCE

Systemic vascular impedance values were calculated from analysis of individual pressure/flow waveform pairs. These pairs were isolated from a sequence of approximately 30 consecutive heartbeats recorded during a period of consistent inter-beat intervals. The impedance moduli of each individual harmonic was then averaged for the entire sequence and the mean spectrum was taken to be typical of the vascular impedance of each animal. Representative analog recordings of pressure and flow waveforms obtained under control conditions are presented in figure 38. Simultaneously recorded waveforms obtained during vasoactive drug administration are presented in figure 39.

Control values of impedance modulus and phase are presented in figure 40. Systemic vascular impedance spectra normalized to peripheral resistance of each individual are represented in
Table 7: Alterations in Cardiovascular Variables in Rats expressed as Percentage of Control Values.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control PTOL</th>
<th>Stand. Dev.</th>
<th>N</th>
<th>PHEN PTOL</th>
<th>Stand. Dev.</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulse Wave Velocity</td>
<td>18.8</td>
<td>1.2</td>
<td>6</td>
<td>21.6</td>
<td>2.5</td>
<td>6</td>
</tr>
<tr>
<td>Heart Rate</td>
<td>65</td>
<td>6</td>
<td>6</td>
<td>65</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Stroke Volume</td>
<td>6.9</td>
<td>2.5</td>
<td>6</td>
<td>6.9</td>
<td>2.5</td>
<td>6</td>
</tr>
<tr>
<td>Mean Arterial Pressure</td>
<td>7.2</td>
<td>1.2</td>
<td>6</td>
<td>9.9</td>
<td>2.5</td>
<td>6</td>
</tr>
<tr>
<td>Peripherial Resistance</td>
<td>-12.8</td>
<td>6</td>
<td>6</td>
<td>-9.2</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Cardiac Output</td>
<td>10.2</td>
<td>6</td>
<td>6</td>
<td>11.3</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Pulmonary Resistance</td>
<td>10.2</td>
<td>6</td>
<td>6</td>
<td>11.3</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

Note: N values are given for each variable.
Figure 36. Changes in Heart Rate, Stroke Volume, and Cardiac Output in Rats, expressed as Percent of Control.
Figure 37. Changes in Pulse Wave Velocity, Peripheral Vascular Resistance, and Mean Arterial Pressure in Rats, expressed as Percent of Control.
Figure 38. Digitized analog recordings of simultaneously measured aortic arch pressure (heavy line) and flow velocity (light line) waveforms in the...

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*Figure 39. Digitized and analog recordings of simultaneous measured aortic arch pressure (heavy line) and flow velocity (right line) waveforms in the rat. Top recording: vasocostricted; bottom recording: vasodilated.*
Figure 40. Systemic Vascular Impedance Modulus and Phase of Individual Rats - Control Values.
figure 41. In all animals, there was a rapid decline in impedance amplitude to a minimum value between the second and fourth harmonics. Following the first minimum, the impedance amplitude rose slowly and showed slight fluctuations through the higher harmonics. In those animals which demonstrated a second minimum, it generally occurred at twice the frequency of the first, but there were no clear second minima in most animals. Phase remained below zero until after the first minimum and then either crossed zero or fluctuated close to zero. Phase continued to fluctuate around zero through the higher harmonics. These findings are indicative of discrete wave reflection effects.

Vasoconstriction (PHEN) impedance and phase spectra are represented in figure 42 with normalized impedance moduli presented in figure 43. In the PHEN treatment group, most animals showed no significant difference in impedance amplitude at the fundamental frequency, however the following fluctuations in impedance were generally greater in amplitude and occurred earlier at higher frequencies than the corresponding control values. In four of the six animals, the first maximum was visible at the second harmonic and a second minimum occurred at twice the frequency of the first. In the remaining two animals, the first minimum occurred at the third and fourth harmonics respectively. Phase approached or crossed zero at a point which coincided with the first minimum and fluctuated widely throughout the higher frequencies. These spectra indicate an earlier return of reflected components to the heart and an increase in impedance in the higher frequency components.
Figure 41. Systemic Vascular Impedance Modulus (Normalized to Peripheral Resistance) and Phase of Individual Rats - Control Values.
Figure 42. Systemic Vascular Impedance Modulus and Phase of Individual Rats - Vasoconstriction Induced by 0.05% Phenylephrine.
Figure 43. Systemic Vascular Impedance Modulus (Normalized to Peripheral Resistance) and Phase of Individual Rats - Vasoconstriction Induced by 0.05% Phenylephrine
Vasodilation (PTOL) impedance moduli and phase are graphically presented in figure 44 and normalized impedance moduli are presented in figure 45. In the PTOL treatment group, all animals experienced an increase in impedance amplitude at the fundamental frequency as compared with control values. In five animals, the impedance modulus fell to a minimum amplitude by the third or fourth harmonic and remained low through the higher frequencies with no clear secondary minima or maxima. In two rats, the first minimum was not apparent until the fifth harmonic and was followed by a second minimum at twice the frequency of the first. In all rats, the minimal impedance amplitude was accompanied by a phase shift toward or crossing zero near the same harmonic. Generally, following the first minimum in impedance, phase fluctuated close to zero through the higher harmonics. The impedance spectra may indicate a delay in the return of wave reflections to the heart.

The mean harmonic impedance amplitudes and phases for all rats were averaged within the control, PHEN, and PTOL groups to produce a group mean impedance spectrum for each treatment. These values are presented in figure 46. Statistical analysis of the impedance amplitude and phase were conducted for each harmonic utilizing a Two-sample t-Test assuming equal variance (P ≤ 0.05). There were statistically significant differences in amplitude at the fundamental harmonic and the third harmonic between the control and PHEN, and at the fundamental harmonic between the control and PTOL groups (P = 0.039, P = 0.037; respectively). There were no statistically significant differences in impedance
Figure 44. Systemic Vascular Impedance Modulus and Phase of Individual Rats - Vasodilation Induced by 0.5 mg Phentolamine
Figure 45. Systemic Vascular Impedance Modulus (Normalized to Peripheral Resistance) and Phase of Individual Rats - Vasodilation Induced by 0.5 mg Phentolamine.

\[ Z_0 / R \]

\[ 0 \]

\[ 0.0 \]

\[ 0.1 \]

\[ 0.2 \]

\[ 0.3 \]

\[ 0.4 \]

\[ 0.5 \]

\[ 0.6 \]

\[ 0.7 \]

\[ 0.8 \]

\[ 0.9 \]

\[ 1.0 \]

\[ 0 \]

\[ 2 \]

\[ 4 \]

\[ 6 \]

\[ 8 \]

\[ 10 \]

\[ 0 \]

\[ 1 \]

\[ 2 \]

\[ 3 \]

\[ 4 \]

\[ 5 \]

\[ 6 \]

\[ 7 \]

\[ 8 \]

\[ 9 \]

\[ 10 \]

\[ 0 \]

\[ 0.5 \]

\[ 1.0 \]

\[ 1.5 \]

\[ 2.0 \]

\[ 2.5 \]

\[ 3.0 \]

\[ -3.0 \]

\[ -2.5 \]

\[ -2.0 \]

\[ -1.5 \]

\[ -1.0 \]

\[ -0.5 \]

\[ 0.0 \]

\[ 0.5 \]

\[ 1.0 \]

\[ 1.5 \]

\[ 2.0 \]

\[ 2.5 \]

\[ 3.0 \]
Figure 46 Mean Systemic Impedance in Rats - Control vs. PHEN and PTOL (Group Mean +/- SE)

* = Statistically Different than Control Impedance Value
amplitudes at any other harmonic. The phase of impedance was statistically different at the fourth harmonic between the control and PHEN groups, but was not statistically different at any other harmonic in either comparison.

PRESSURE PULSE AMPLIFICATION AND DISTORTION
In the control group, comparison of simultaneously recorded aortic arch and abdominal aortic pressures showed peripheral pulse wave amplification and distortion. A representative tracing of a paired pressure pulse and the corresponding Fourier analysis is presented in figure 47. All aortic arch pressure tracings showed a clearly identified incisura and apparent dicrotic wave. In all individuals, there was an increase in pulsatile pressure in the abdominal aorta (mean value 40.6 ± 5.6 mmHg) over the aortic arch (mean value 32.6 ± 5.1 mmHg), and the peripheral waveform demonstrated a more rapid systolic rise. The incisura was not apparent and the dicrotic wave was smoothed in the peripheral recordings of all animals. Harmonic analyses demonstrated peripheral amplification with fluctuations in amplitude throughout the frequencies of both pulses.

In the PHEN group, all individuals exhibited a slight increase in aortic arch pulsatile pressure (PParch mean = 39.8 ± 20.4 mmHg) as compared with control values and an increase in pressure pulse amplification. Abdominal aortic pulse pressures increased to a mean value of 56.9 ± 20.7 mmHg. A representative paired pressure pulse tracing and the corresponding Fourier analysis are presented in figure 48. Individual peripheral pulse amplification
Figure 47. Simultaneously Recorded Aortic Arch and Abdominal Aortic Pressure in a Rat - Control
Top Graph - Digitized Recording, Bottom Graph - Fourier analysis of Paired Pressure Pulse

Mean Measurement Error per 100 mmHg = 1%
Figure 48. Simultaneously Recorded Aortic Arch and Abdominal Aortic Pressure in a Rat -
Vasoconstriction Induced by 0.05% Phenylephrine
Top Graph - Digitized Recording, Bottom Graph - Fourier Analysis of Paired Pressure Pulse

- Solid - Aortic Arch Pressure
- Hatched - Abdominal Aortic Pressure

Mean Measurement Error per 100 mmHg = 1%
was usually accompanied by a steeper rise and more rapid runoff in the early portion of diastole as compared with the central pulse. The dicrotic wave was generally larger in amplitude and later in diastole than the pattern observed during control measurements. In the aortic arch, the increase in peak systolic pressure was accompanied by a sharper decline in pressure before the closure of the aortic valve and the dicrotic wave was both larger and appeared earlier in diastole than the corresponding control. Harmonic analysis illustrated pronounced peripheral pulse amplification at the first harmonic frequency and large secondary maxima in both pulses at the third or fourth harmonic in all animals. The second maximum was followed by small fluctuations in moduli in the higher harmonics.

Figure 49 illustrates a paired aortic arch and abdominal pressure analog recording and the Fourier analysis of a representative animal under PTOL conditions. Mean aortic arch pulsatile pressures were less than control values (28.7 ± 7.1 mmHg) and mean abdominal pulsatile pressures declined to 25.3 ± 8.4 mmHg. There was a visible loss of peripheral pulse amplification and distortion. Generally, central and peripheral waveforms were very similar in appearance with a slightly greater peak systolic pressure centrally. Systolic rise time was similar and there was no apparent incisura or dicrotic wave in either tracing. The Fourier analysis demonstrated a single peak at the fundamental frequency and a decline in amplitude to very small values in the remaining harmonics. There were no significant fluctuations in moduli in either waveform.
Figure 49. Simultaneously Recorded Aortic Arch and Abdominal Aortic Pressure in a Rat - Vasodilation Induced by 0.5 mg Phentolamine
Top Graph - Digitized Recording, Bottom Graph - Fourier Analysis of Paired Pressure Pulse

Mean Measurement Error per 100 mmHg - 1%
FLOW VELOCITY WAVEFORMS

A representative pair of control aortic arch pressure and flow waveforms are presented in figure 50. The flow waveforms are characteristic of the recordings obtained in all rats from this group with slight differences in the magnitude of the flow incisura and alterations in ejection duration with heart rate. The mean flow velocity of the PHEN animals (mean = 21.6 ± 5.8 cm/sec) was not statistically significantly different (at P ≤ 0.05) from control values (mean = 25.8 ± 8.7 cm/sec). The PTOL animals demonstrated a slight decrease in flow velocity to 22.3 ± 13.0 cm/sec, but this decline was not significantly different at P ≤ 0.05.

ARTERIAL BIOMECHANICS

Figure 51 illustrates the circumferential stress-strain behavior and $E_{inc}$ of the rat abdominal aorta. At a mean physiological pressure of 90 mmHg (~12.3 kPa) the $E_{inc}$ modulus was 400 kPa. The stress-strain curve for the abdominal aorta in rats illustrated a gradual increase in relative stiffness with increasing distending pressure. This curve shape is more typical of mammalian central arterial behavior and is punctuated by an earlier increase in stiffness than that observed in the mouse. An increase in the slope of the stress-strain curve is observed at about 12 kPa (90 mmHg), appropriate to the mean arterial pressure range of this animal. This curve indicates mechanical behavior which is consistent with the measured pulse wave velocities and mean arterial pressures. A graphical comparison of the stress-strain relationships and elastic moduli of both species is
Figure 50. Simultaneously Recorded Aortic Arch Pressure and Flow Velocity Waveforms in a Rat.
Figure 51. Circumferential Stress-Strain Relationship (+/- sd), and Elastic Modulus as a Function of Distending Pressure (+/- sd), in the Abdominal Aorta of Rats.
The predicted velocity of pressure pulse wave propagation ($C_o$) and predicted pressure pulse transit time expressed as percentage of cardiac cycle ($\%CC_{pred}$) were calculated from the biomechanical measurements. These values, as well as the measured values, are presented in table 8. The mean measured $C$ (mean = $4.42 \pm 0.69$ m/s) was 30% greater than the $C_o$ (mean = $3.64 \pm 0.85$). The measured $\%CC$ (mean = $12.2 \pm 1.4$) were 24% less than the $\%CC_{pred}$ ($16.0 \pm 4.2\%$). There was no significant difference between measured and predicted values for either variable at $P \leq 0.05$.

**AORTIC TISSUE COMPOSITION**

Figure 53 portrays the anatomical composition of the abdominal aortic wall in the rat. The top photograph is a cross-sectional segment, with a closeup of a wall portion presented below. In both, the internal elastic lamella is clearly visible at the interface of the tunica intima and luminal space, and the external surface is demarcated by adventitia. Approximately nine concentric elastic lamellae are visible. The abdominal aortic wall is relatively thick, with a slightly higher lamellae/thickness ratio (0.41) than that measured in the mice (0.33). The composition of the abdominal aortic wall is typical of mammalian arteries and is consistent with biomechanical stress testing results.

**ALLOMETRIC PREDICTIONS OF CARDIOVASCULAR VARIABLES**

Allometrically predicted and mean measured values of $L$, $HR$, $SV$, $
Figure 52. Circumferential Stress-Strain Relationship, and Elastic Modulus, as a Function of Distending Pressure in the Abdominal Aorta of Rats and Mice
Table 8 Pressure Wave Velocity and Transit Time Calculated from Mechanical Properties versus Measured Values in Rats

<table>
<thead>
<tr>
<th>Animal Number</th>
<th>Measured C (m/s)</th>
<th>Predicted C (m/s)</th>
<th>Measured %CC</th>
<th>Predicted %CC</th>
</tr>
</thead>
<tbody>
<tr>
<td>504</td>
<td>3.79</td>
<td>2.16</td>
<td>13.1</td>
<td>22.7</td>
</tr>
<tr>
<td>507</td>
<td>5.33</td>
<td>3.97</td>
<td>10.5</td>
<td>14.2</td>
</tr>
<tr>
<td>517</td>
<td>4.74</td>
<td>3.78</td>
<td>11.7</td>
<td>14.3</td>
</tr>
<tr>
<td>519</td>
<td>3.66</td>
<td>4</td>
<td>14.1</td>
<td>11.7</td>
</tr>
<tr>
<td>602</td>
<td>4.57</td>
<td>4.28</td>
<td>11.8</td>
<td>17.1</td>
</tr>
</tbody>
</table>

| Mean          | 4.42             | 3.64              | 12.2         | 16            |
| Standard Deviation | 0.69             | 0.85              | 1.4          | 4.2           |
Figure 53. The abdominal aorta of a rat. Cross-sections fixed at 0 distending pressure. Top photograph - Whole aorta, 220x magnification. Bottom photograph - Wall segment, 880x magnification.
and CO of control animals are presented in table 9. The cardiac variables are also represented graphically in figure 54. All calculated values were based on the mean body mass of the control group (0.5725 kg). The mean measured L of 0.0995 meters was 10% less than the predicted value of 0.1096 meters, and HR (6.05 beats per second) was greater (4.52 beats per second) by 34%. Measured SV was less than the predicted value by 14% at 0.5013 and 0.5706 mls/beat/kg; respectively. CO was 0.181 L/min/kg, 17% greater than the predicted value of 0.155 L/min/kg. Although the discrepancies between the predicted and measured CO and SV are small, the relative contribution of HR is much greater than that which is predicted. The smaller SV contribution to CO appears to be partially compensated for by the increased resting heart rate.
Table 9. Allometrically Predicted versus Measured Cardiovascular Variables in the Rat.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Predicted Value</th>
<th>Measured Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aortic Length (meters)</td>
<td>0.1096</td>
<td>0.0995</td>
</tr>
<tr>
<td>Heart Rate (beats/second)</td>
<td>4.52</td>
<td>6.05</td>
</tr>
<tr>
<td>Pulse Wave Velocity</td>
<td>4.4</td>
<td>4.2</td>
</tr>
<tr>
<td>Length/Lambda</td>
<td>0.11</td>
<td>0.14</td>
</tr>
<tr>
<td>Stroke Volume (mls/beat/kg)</td>
<td>0.571</td>
<td>0.501</td>
</tr>
<tr>
<td>Cardiac Output (L/min/kg)</td>
<td>0.155</td>
<td>0.181</td>
</tr>
</tbody>
</table>
Figure 54: Allometrically Predicted versus Measured Cardiovascular Variables in the Rat.

- Predicted Cardiac Output ($E=187, P=0.87$)
- Measured Cardiac Output (L/min/kg)
- Predicted Stroke Volume ($E=0.66, P=1.05$)
- Measured Stroke Volume (mL/s/kg)
- Predicted Heart Rate ($E=3.93, P=0.25$)
- Measured Heart Rate (b/s)

(Allometric values from Holt et al., 1968)
Chapter IV.
DISCUSSION

The properties of pressure wave transmission and its effect on ventricular/vascular coupling in the mammalian circulation are commonly represented by Tubular models of hemodynamics. In 1960, McDonald described the arterial tree as an asymmetric-T whose long limb represented the combined arterial terminations of the aorta and lower body vasculature and the shorter limb depicted the resultant terminations of the upper body. Wave reflections, due primarily to the high arteriolar tone within these vascular beds, were shown not only to determine the contour of the arterial pulse but also to decrease systemic input impedance (McDonald, 1960). This theory was advanced by Taylor and O’Rourke who explained both ascending aortic impedance patterns and the characteristics of the arterial pulse on the basis of four individual peripheral reflecting sites. These points were identified as two separate sites in the upper body, distinguished as the brachiocephalic and subclavian arterial beds, and two lower body sites, the descending aorta and lower body vascular terminations (Taylor, 1966; O’Rourke and Taylor, 1966, 1967; O’Rourke, 1965, 1967).

Utilizing multi-branched tubular models, Taylor (1957) illustrated that input impedance is affected by both the spatial dispersion of the reflecting sites and the wave velocity (Taylor, 1957a, 1957b, 1965, 19661, 1966b). When reflecting sites are grouped closely together, the total reflection coefficient at low
frequencies is almost as high as it is for each individual site, hence the sum of reflections is "seen" at a single point. When there is a greater dispersion between individual locations, discrete wave reflection effects are less visible in both the pulse profile and input impedance spectrum. Wave velocity (C), determined by the elastic nature of the tubes, influences the impedance spectra as a result of differences in the magnitude and timing of the return of reflected waves to the input source. If the effective tube length and the frequency of the pulsatile pressure input are matched correctly, a minimum of impedance modulus occurs at a frequency where the distance to the reflecting site is approximately one-quarter of the fundamental wavelength.

In mammalian studies, these same characteristics of wave propagation have been demonstrated and are known to strongly influence both the arterial pulse profile and the input impedance of the arterial tree (Nichols et al., 1977; O'Rourke and Avolio, 1980). While the eccentric location of the heart results in a disproportionate length to the upper and lower body reflecting sites, C is greater in the lower body vessels as a result of geometric taper and decreased arterial distensibility. In many mammals, this results in an almost simultaneous return of wave reflections from each of the vascular beds to the heart. Thus, the impedance fluctuations appear as if the reflections arose at a single site which, for convenience, is regarded as being located in the lower body.
Since ascending aortic pressure pulse contour and amplitude is largely determined by systolic duration, mean arterial pressure (MAP), peripheral arterial resistance (Rs), and C, the pulse shape is very similar among mammals (Milnor, 1990; Nichols and O’Rourke, 1990). It is characterized by a rounded systolic peak, an incisura created by aortic valve closure, and a dicrotic wave. The most visible differences in pulse contour between larger animals is noted in the dicrotic wave which results primarily from peripheral wave reflection and is therefore, affected by body shape (Avolio et al., 1982; Nichols and O’Rourke, 1990). Discrete wave reflection effects are most apparent in animals that have large peripheral vascular beds (e.g. the kangaroo) and least visible in animals whose body design promote a wide dispersion of muscle groups (e.g. snakes) (Avolio et al., 1982; Avolio, Nichols and O’Rourke, 1985).

In some small mammals, birds, and in aging humans, the pressure pulse contour assumes a much different shape. Peak pressure is reached later in systole and there is an almost exponential decline in pressure following the incisura. These effects have been shown to result from increased arterial stiffness in humans, and a high pulse wave velocity in birds (Nichols and O’Rourke, 1990). However, in many small mammals the early return of peripheral wave reflections is a consequence of a short aortic length relative to heart rate. In each case, the reflected waves return to the heart during systole and combine with the pulse peak. Consequently, the central pressure wave contour is nearly identical to that measured in the abdominal aorta.
Length/Heart Rate Relationships

Many researchers have attempted to utilize the concept of a single "apparent" peripheral reflecting site to explain both the impedance patterns and the arterial pulse contours observed in the proximal aorta. Since $C$ is a function of the viscoelastic properties of the arterial vessels, which are similar in different mammals, this implies that the minimization of systemic arterial impedance through wave interactions is a function of body size ($L$) and resting heart rate ($f_o$) (McDonald, 1960; Milnor, 1979). As has been previously discussed, Tubular models of hemodynamics predict that cardiac function, with regard to cardiac energy expenditure, is optimal when $L$ and $f_o$ are matched so the first minimum of impedance modulus and phase crossover occurs at a frequency where $L/\lambda$ is equal to 0.25 (one-quarter wavelength).

When $L$ and $f_o$ are optimally matched, a favorable correlation between the first minimal value in ascending aortic impedance and the maximum harmonic magnitude of left ventricular flow is established. Since the duration of ejection increases with decreases in heart rate, this advantageous relationship can usually be maintained as body size (aortic length) decreases. However, allometric calculations illustrate mass dependent decreases in aortic length which occur at a greater exponential rate than the corresponding increases in cardiac frequency (-0.33 and 0.25; respectively). This predictive concept indicates that at very small masses, cardiac frequency may be inappropriately low with regard to aortic length and the $1/4 \lambda$ correlation.
Avolio (1976) demonstrated this in guinea pigs where the frequency of the first minimum of impedance modulus and phase crossover occurs at a higher frequency than in larger animals and is shifted with respect to the maximum flow harmonic. He measured the first impedance minimum at about 11 Hz as compared with 4.5 Hz in the rabbit and 6 Hz in medium sized dogs (Avolio et al., 1976; Milnor, 1979). These values correspond to L/λ's of 0.14, 0.19 and 0.17; respectively. Milnor (1979) reported a similar high frequency impedance minimum in rats, demonstrating its occurrence at 12 Hz which corresponds to a L/λ of approximately 0.14.

**Ventricular/Vascular Coupling - Input Impedance Spectra**

In this study, impedance measurements made in open-thorax, ventilated rats, were characteristic of small mammals. The first minimum of impedance modulus and phase crossover was observed in most rats between 12 and 18 Hz. Although there was generally no apparent second minimum, the higher harmonic frequencies were characterized by small fluctuations in magnitude. The \(1/4\) \(λ\) reflecting site, calculated from the mean frequency of the first minimum of impedance (figure 46), corresponds to a peripheral reflecting site located approximately 9 cm from the recording site in the aortic arch. This value is equivalent to the L of 9.9 cm measured from the aortic value to the iliac bifurcation. In accordance with these measurements, the major peripheral reflecting site would be located at the iliac bifurcation.

When the rats were vasoconstricted, the first minimum of
impedance and phase crossover was usually observed at about the same frequency as it was under control conditions but the magnitude was generally lower. The most notable difference was an increase in the impedance magnitude of the higher harmonic frequencies accompanied by greater fluctuations in phase. The group mean spectrum showed a statistically significant decline in impedance at the fundamental harmonic, with the first minimum occurring at 13 Hz, followed by a small maximum and phase crossover at the third harmonic (figure 46). There was a mean increase in peripheral vascular resistance (Rs) of 72% in this group, accompanied by an increase in C to 6.92 m/s from a control value of 4.19 m/s. There was only a small increase in heart rate to 6.47 b/s from 6.05 b/s (tables 6 and 7). The large increase in C, combined with the small change in fQ, resulted in an earlier return of wave reflections to the heart and an increase in the impedance amplitudes of the higher harmonics. In this group, the mean L was calculated to be 9.6 cm (anatomically measured mean L was 9.85 cm) indicating that the major peripheral reflecting site was, as in the control group, located at the iliac bifurcation. While the location of the major site of reflection did not appear to change, the strength and timing of reflections was altered by the increase in peripheral vascular resistance.

Under conditions of peripheral vasodilation, the predicted first minimum should have been visible at about 9 Hz in most rats. However, the first minimum of impedance and phase crossover was not generally evident until about 13 Hz. The impedance minima were observed at approximately the same frequency at which it
 occurred under control conditions in the individual spectra (figure 45). There was a general decline in impedance magnitude in the higher frequencies and, in most animals, phase remained negative or displayed a high frequency crossover. Although peripheral vascular tone was diminished, as was evidenced by the decline in Rs of 36% and MAP of about 50%, there was only a slight decline in C from the control velocity. The mean heart rate (6.69 b/s) was not statistically significantly different from control (tables 6 and 7). Consequently, the results of these physiological alterations were most visible not in the timing of reflections, but in their magnitude. While wave reflections reached the heart at about the same time, discrete effects were not as visible in the impedance spectrum as a result of the reduction in the reflection coefficient. In this group, the major peripheral reflecting site was calculated to be about 8 cm from the central recording site. This corresponds to a point midway between the right renal artery and the iliac bifurcation in rats.

The impedance spectra measured in open-thorax, ventilated mice were markedly different from those observed in the rats in this study. In the control animals, there was a rapid decrease in impedance amplitude from the first harmonic, followed by a continual, gradual fall in impedance amplitude with increasing frequency (figure 21). There was no clear first minimum and no phase crossover at any frequency studied. The $1/4 \lambda$ reflecting site could not be calculated for mice under these conditions since the first impedance minimum was not identifiable. However, if the measured aortic valve - iliac bifurcation length is
assumed to be equivalent to the $1/4 \lambda$ in these mice, the first minimum of impedance would not be predicted to be observed until a point above the 20th harmonic. At this high frequency, the influence of wave interactions would be insignificant and would contribute little to the reduction of systemic input impedance.

In vasoconstricted mice, there were statistically significant increases in $f_0$, $C$, and $Rs$, indicating a potential difference in both the timing and magnitude of peripheral reflections (tables 1 and 2). While these physiological alterations were evident as a slight decline in the magnitude of impedance in the lower harmonic frequencies of some animals, the mean magnitude of impedance and phase were not significantly changed from control values at any frequency (figure 26). There were no clear minima or maxima in any individual or in the mean spectra. The phase remained negative at all frequencies in all individuals, and the mean spectra were not statistically different from control patterns. In two animals, there were small fluctuations in impedance modulus in the higher harmonics, but these were not accompanied by a phase crossover (figure 23). In this treatment group, despite the large increase in peripheral vascular tone and in heart rate, neither the individual or the mean impedance spectra indicate any significant discrete wave reflection effects on systemic impedance.

The vasodilated mice experienced an average decrease in $Rs$ of 41%, but there was no statistically significant difference in MAP or $C$ (tables 1 and 2). While the impedance spectra in these
animals were largely unchanged from control conditions, three mice showed a minimum of impedance by the second harmonic with little or no fluctuations in the higher harmonics (figure 25). In the mean spectra, there was a statistically significant increase in mean impedance modulus at the second and third harmonics as compared with control values (figure 26). This was accompanied by a lower mean phase at the second harmonic than the control value. The significance of this is not clear since the general phase pattern is similar to the control conditions. In these animals, the impedance spectra do not indicate any discrete wave transmission effects; however, the increase in the magnitude of impedance at the second and third harmonics may indicate alterations in ventricular ejection properties. The mean SV of these animals nearly doubled in response to the decline in peripheral Rs, thus maintaining MAP and altering the ejection duration.

Windkessel Predictions and Murine Hemodynamics

Previous investigators have demonstrated that while many of the details of aortic input impedance spectra cannot be reproduced by Windkessel models, the basic features are reasonably represented (Burkhoff et al., 1988; Fitchett, 1991). There are many variations of the simple Windkessel design but the three-element model, which encompasses an arterial compliance element in addition to the characteristic impedance and arterial resistance components, has been shown to be the most representative of ventricular/vascular coupling. Burkhoff et al., (1988) utilized this model to predict ventricular afterload in dogs, and although
individual waveforms were not realistically represented, the impedance modulus began at the same magnitude as that computed from ascending aortic pressure and flow. The impedance modulus settled at the same higher frequency values in both cases; however, there were no minima or maxima present in the predicted spectra. Comparison of the real and predicted phase relationships illustrated a primary difference in the crossover frequency. In the measured spectrum the phase crossed 0 at a frequency which corresponded with the first maximum and continued to rise, whereas the predicted phase began at 0°, declined to a more negative value at a lower frequency, and was followed by a monotonic rise toward 0°. Burkhoff's comparison suggested that small fluctuations of impedance may not have a significant effect on ventricular/vascular coupling and that the system is primarily dependent on the characteristic impedance, arterial resistance, and arterial compliance. In this view, the Windkessel model provides an adequate description of the arterial system and a reasonably accurate prediction of impedance magnitude in the lower harmonics.

To demonstrate the correlation between the measured impedance of mice and that which is predicted by the three-element model, the average impedance was calculated from the mean values for each treatment group. These values were then used to compute the predicted impedance spectra. Both the measured and predicted values were standardized to the calculated characteristic impedance. A comparison of the mean measured and predicted spectra is presented in figure 55.
Figure 55. Mean Systemic Vascular Impedance in Mice (Normalized to Characteristic Impedance)

Average Measured Values versus 3 - Component Windkessel Model Predictions for Control, PHEN and PTOL Treatment Groups

- Control, HR 7.45 Hz
- Predicted Control, HR 7.45 Hz
- Vasodilation, HR 7.62 Hz
- Predicted Vasodilation, HR 7.62 Hz
- Vasoconstriction, HR 9.37 Hz
- Predicted Vasoconstriction, HR 9.37 Hz

Figure 55. Mean Systemic Vascular Impedance in Mice (Normalized to Characteristic Impedance)
Under control conditions, the measured impedance was less than the predicted values throughout the analyzed frequency spectrum. There were no minima or maxima in impedance modulus in either the predicted or observed spectra. Phase relationships were similar in that they began at 0 radians, declined to a minimum value and settled. The predicted minimum phase was reached at the first harmonic, while the measured minimum phase was reached at the second, and it remained more negative.

In the vasodilated group, the predicted and measured magnitudes of impedance modulus were nearly identical at the first harmonic. However, the measured values continued to decline with increasing frequency while the predicted values were essentially constant from the second through the fifth harmonics. Phase patterns in the predictive model were identical to the predicted control phase. The measured phase values were more negative than the measured control and displayed a minimum at the first harmonic followed by a small, gradual rise toward zero.

When the mice were vasoconstricted, distinct differences between the predicted and measured spectra became apparent. In the predictive model, the impedance modulus fell rapidly from the characteristic impedance value to a low magnitude at the fundamental harmonic followed by a very gradual decline through the higher frequencies. The measured spectrum demonstrated a greater than predicted impedance magnitude at the first harmonic
followed by lower magnitudes in the higher frequencies. The predicted phase pattern was nearly identical to the predicted control conditions. However, while the measured phase was less negative than the predicted values at all frequencies, it was more negative than the measured control. In addition, the phase showed small fluctuations and a slight rise at the fourth harmonic followed by a decline at the fifth. These differences illustrate potential reflection effects in the mean measured spectrum as compared with the predicted spectrum of a functional Windkessel.

This comparison illustrates that the three-element Windkessel model does not adequately represent the aortic input impedance spectra of mice. While under control conditions neither the measured or predictive impedance patterns are characteristic of discrete wave reflection effects, the measured spectra is distinctly different from the predictive model. In addition, when the mice are vasoconstricted the Windkessel model underestimated the impedance modulus at the fundamental harmonic. These results indicate that it is not appropriate to classify mice as functional Windkessels under any of the conditions explored in this study.

**Arterial Flow Profiles**

The contour of the aortic flow wave is known to be determined primarily by the properties of ventricular ejection which are modified by preload, ventricular characteristics, and the impedance of the arterial tree. In small mammals, despite the
relatively longer duration of ejection, the wave contour has been shown to be similar to that seen in larger species. Flow velocity waves recorded in guinea pigs and rats illustrate a typical pattern of ejection and peak velocities which are comparable to other larger mammals (Avolio, 1976; Milnor, 1979). These waveforms are characterized by a rapid rise to peak velocity followed by a sharp decline and brief backflow which coincides with aortic valve closure.

In this investigation, the aortic arch flow waveforms recorded in rats (figure 14) were typical of those seen in other mammals and the ejection durations and peak velocities measured under control conditions were comparable to other reported values (Milnor, 1979; Bishop, 1980). In contrast, although mice exhibited similar peak ejection velocities and ejection durations (as percent of cardiac cycle) which were expected for a heart rate of 450 b/m, there was an absence of backflow in the flow waveforms of the control animals (figure 13). This finding is indicative of an increase in central aortic distensibility and greater cushioning of the ejection volume as compared with other mammals. When the mice were vasoconstricted, there was a slight increase in the ejection duration, an increase in heart rate, and an increase in C, but backflow remained indistinct. Although alterations in other physiological variables indicated an increased in peripheral vessel stiffness, the central arterial vasculature appeared to remain highly distensible.
Arterial Pressure Pulse Profiles

While quantitative reports of cardiovascular variables in rats, including arterial pressures, are limited, the measurements obtained in this study are comparable to values determined by other investigators. MAP and pressure pulses (PP) recorded under anesthesia, but prior to open-thoracic manipulations, were not statistically different (at P < 0.05) than those measured after the chest was incised. The average MAP was 92.7 ± 18.3 mmHg while the mean aortic PP was measured at 32.6 ± 5.1 mmHg. These MAP's are similar to average reported values of 92.72 mmHg (Bishop, 1980) and 103.6 mmHg (Mosberg et al., 1992) measured in unanesthetized rats. Although the pulsatile pressures recorded here are slightly greater than other reported values (by 6—8 mmHg), this difference is one of calculation. In this study, to facilitate the computerized evaluation of pulsatile pressures, PP was defined as the difference between peak systolic and minimum diastolic pressures whereas reported values are generally based on the difference between the means of each (Bishop, 1980; Mosberg et al, 1992). The mean f_o, mean Rs and mean measured C were all within the expected range (calculated allometrically) at about 365 b/m, 45,000 dyn-s cm^{-5} kg^{-1}, and 4.2 m/s; respectively.

The pressure waveforms recorded in the control rats were similar to both those described in recent literature, and to those described in larger mammals (figure 11) (Bishop, 1980). In most anesthetized rats, the aortic arch pulse contour was characterized by a rounded systolic peak, a demonstrated incisura and a visible dicrotic wave. Differences between the pulse...
profile of individuals were most notable in the size and timing of the dicrotic wave relative to the duration of diastole. This was attributed to small differences in the state of peripheral vascular tone, C and f0. Peripherally, there was generally systolic peaking apparent while centrally, there was a loss of the incisura and a late or absent dicrotic wave in all waveforms. These patterns are indicative of discrete wave summation between the incident wave and its reflected components in rats under control conditions.

In vasoconstricted rats, both peak systolic pressures and PP were increased centrally. There was generally a sharper systolic contour and either an earlier, larger dicrotic wave, which occurred almost immediately after closure of the aortic valve, or the dicrotic wave was not visible. The absence of the dicrotic wave occurred in instances of greatly increased C and Rs, and was accompanied by large increases in peak systolic pressure which are suggestive of a very early return of wave reflections. Peripherally, PP was significantly increased and there was often a visible late dicrotic wave. These observations can be explained by the increased magnitude of wave reflections and their earlier systolic return to the heart.

Under vasodilated conditions, both the central and peripheral PP, and the MAP of rats declined. In both pulses, the systolic contour became more rounded, and there was a loss of both the dicrotic wave and the incisura in most animals. The central and peripheral pulses generally appeared to be almost identical in
contour. In these animals, the arterial pulse waveform indicates a decline in both the magnitude and timing of peripheral wave reflections.

The arterial pulse contours observed in anesthetized mice (figure 11) were significantly different than those recorded in the rats in the present study, and in other small animals (e.g. birds, Woodbury and Hamilton, in Nichols and O'Rourke, 1990). While the sharp systolic peaks and exponential diastolic pressure decline observed in mice in early recordings made by Woodbury and Hamilton (1937) were interpreted to be caused by the early return of wave reflections, they reported central systolic pressures of 160 mmHg and diastolic pressures of 120 mmHg at an f_o of 590 b/m (Nichols and O'Rourke, 1990). Their reported heart rate is, although high, within more recently reported ranges, but the MAP is much higher than that measured here and by other investigators. In a study conducted by Rockman et al., (1991), a 7-day average aortic root MAP of 47.0 ± 13.0 mmHg (f_o averaged 360 b/m) was measured in awake, unrestrained mice. In addition, Wetterlin (1977) reported an average MAP, recorded under anesthesia via carotid artery cannulation, of 57.10 ± 7.0 mmHg at a f_o of 386 ± 62.0 b/m.

In this investigation the mean MAP of 59.6 ± 11.3 mmHg recorded in anesthetized, but closed-thorax, mice was not statistically different (P ≤ 0.05) from the pressure of the open-thorax animals. Centrally, the mean PP was not significantly different than that measured in rats. However, the mean peripheral PP in
the mice was lower than the PP measured centrally. The waveforms of most animals were characterized by a slightly more gradual rise to peak systolic pressure, an absence of a clear incisura, and a gradual diastolic decline without secondary fluctuations. In a few individuals, very small amplitude fluctuations were observed in late diastole, but a clear dicrotic wave was not identifiable. Peripherally, the pulse demonstrated a rounding of the systolic peak and a nearly exponential diastolic decline. Given the slow C and the low MAP, it is likely that the degree of wave reflection is minimal and their return to the heart delayed so that there is little influence on the central pressure contour.

When the mice were vasoconstricted, MAP, central PP and peripheral PP, C and $f_0$ increased substantially. In this case, the PP alterations must be considered individually since peripheral pressure recordings were nearly impossible to obtain. The large increase in Rs usually caused the arterial walls to collapse around the cannula and the arterial pressure signal was subsequently lost. In the two individuals in which peripheral pressure recordings were obtained, there was some evidence of peaking and a less gradual decline in diastolic pressure. Centrally, these animals demonstrated a small diastolic wave which was visible during the first one-third of diastole. There was still no evidence of an incisura. It is possible that, under conditions of extreme vasoconstriction and greatly increased heart rate, mice may experience some discrete wave reflection effects as evidenced by alterations in the central and peripheral
arterial wave contours. However, even under these conditions there was still no indication of an inappropriately early return of wave reflections so that systolic summation of the incident and reflected components occurred.

In vasodilated mice, the average MAP and central PP were nearly identical to control values, however, peripheral PP declined by about 4 mmHg. While $f_0$ was largely unchanged from control levels, MAP was maintained at control levels primarily by large increases in stroke volume. $C$ declined slightly under these conditions and the central and peripheral pulse waveforms were essentially identical. At each location, there was an exponential decline in diastolic pressure and no incisura or dicrotic wave. In these waveforms there was no evidence of discrete wave reflection effects.

Central Arterial Biomechanics
Since the propagation velocity of the arterial wave is primarily determined by the viscoelastic properties of the aorta, the stress-strain relationships were determined in each of the study species. The abdominal aorta was chosen for examination because the short segment length of the ascending aorta in mice made it technically impossible to isolate. While it is known that the mammalian abdominal aorta is characterized by a gradual taper in diameter and a decrease in distensibility as compared with the proximal aorta, it is a well-studied vessel in rats and provides a basis for direct comparison with mice.
Anatomically, the abdominal aorta of the mouse was thin walled, had an average of 3 elastic lamellae, and a lamellae:wall thickness ratio of 0.33. In the histological sections, collagen fibers were sparse and irregularly distributed within the wall (figure 34). In comparison, the rat abdominal aorta was thicker, had an average of 9 lamellae and a greater lamellae:wall thickness ratio of 0.41 (figure 53). Collagen occurred throughout the wall as well as within the adventitia. While there are no reported data for the mouse, the rat findings concur with those of Gibbons and Shadwick (1989). Their examination of the lower abdominal aorta showed the presence of organized collagen fibers throughout the wall, an average of 11 lamellae, and a lamellae:wall thickness ratio of approximately 0.39.

The biomechanical tests of the stress-strain relationships and calculated $E_{inc}$ of the upper abdominal aorta in the study rats were typical of larger mammals (figure 51). There was a gradual rise in strain proportional to stress until high in the physiologically normal pressure range and then a relatively rapid increase at a stress of about 16 kPa (this corresponds to an expression of 200 mmHg). At this stress level the corresponding strain value was approximately 0.18. Calculations of the quasi-static elastic modulus illustrated that at mean physiological pressure (about 12 kPa or 90 mmHg), the $E_{inc}$ was approximately 400 kPa. Gibbons and Shadwick (1989) reported an aortic $E_{inc}$ measured at the iliac bifurcation in rats, of 500 kPa. This slightly increased value reflects the lower anatomical measurement site.
The measurement of abdominal aortic characteristics in mice demonstrated some striking differences in distensibility (figure 33). The stress-strain curves were distinguished by a much more gradual increase in the relationship and no abrupt increase in stress until about 15 kPa (200 mmHg). At this stress, the circumferential strain was about 0.28. The quasi-static $E_{inc}$, measured at the mean physiological pressure of 60 mmHg (8 kPa), was approximately 250 kPa, and the average slope of the pressure-$E_{inc}$ curve was less than that of the rat. These measurements indicate a much greater aortic distensibility in the mouse than in larger mammals and provides support for both the low MAP and the slower measured C by which this animal is characterized.

$L/\lambda$ Ratio as an Indicator of Ventricular/Vascular Coupling

While the ideal timing of peripheral wave reflections may be an important teleologic determinant of the relationship between L and $f_0$ in mammals, the use of the $L/\lambda$ ratio as an indicator of wave transmission effects may be not be appropriate. The distance to the peripheral reflecting site (L) is defined by the $1/4 \lambda$ concept in tubular models and is calculated with respect to C and the first minimum of the input impedance. This L is then utilized to identify the $L/\lambda$ ratio based on the fundamental heart rate (McDonald, 1960; Bergel, 1961; O’Rourke and Taylor, 1967; Anliker, 1968; Li et al., 1981; Callaghan et al., 1984). Since the relationship between C / $f_0$ determines $\lambda$, independent alterations in vascular tone or $f_0$ can significantly alter the fundamental $\lambda$ while the impedance minimum may not shift in the frequency domain.
Table 10. Comparison of the mean L/A ratios calculated for Control, PHEN and PTOL Treatment Conditions in Rats and Mice

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PHEN</th>
<th>PTOL</th>
</tr>
</thead>
<tbody>
<tr>
<td>L/A (Rat)</td>
<td>0.106</td>
<td>0.1219</td>
<td>0.02</td>
</tr>
<tr>
<td>L/A (Mouse)</td>
<td>0.1463</td>
<td>0.1508</td>
<td>0.03</td>
</tr>
</tbody>
</table>

- = Statistically different than Control at P < 0.05

Mean HR (b/min)
Mean Rs (dyn s/cm²/kg)
Pulse Wave Velocity (m/s)
Figure 56. Relationship Between Length / Lambda and Peripheral Resistance (Control versus Vasoconstriction / Vasodilation Treatment Conditions: Individual Mice and Rats)
Figure 57. Relationship Between Central and Peripheral Pulse Pressure Differential and Length/Lambda Ratio, Control vs. Vasoconstriction / Vasodilation Treatment Conditions (Individual Mice and Rats)
contradiction between the observed reflection effects and those indicated by the increase in the L/λ ratio in all individuals.

In contrast, an increase in peripheral vasoconstriction (Rs increased 72%) resulted in a decrease in the L/λ ratio of rats to 0.1008. In these animals, the mean increase in C was much greater than the alterations in f₀. Thus, λ was significantly lengthened. In the impedance spectra, the frequency of the first minimum of impedance was not significantly different than the control value in most individuals; however, alterations in the magnitudes of the impedance modulus were suggestive of increased wave reflection. The arterial pulse profiles also showed a significant increase in wave reflection effects. The data observed under vasoconstrictive conditions support an increase in wave reflection effects while the mean L/λ indicates a decrease.

In mice, under control conditions, there was less variation in the individually calculated L/λ ratios. However, this is primarily a result of the determination of L. In all animals, there were no clear minima of impedance and therefore, L was calculated from the measured distance between the aortic valve and the iliac bifurcation. L/λ ratios determined under control conditions were always indicative of an absence of discrete wave reflection effects, and were supported by both the impedance spectra and arterial pulse profiles.

When the mice were vasodilated there was a mean decrease in peripheral vascular resistance of 40%, but the calculated L/λ
rose to 0.1187. This ratio indicates a small increase in the potential for discrete wave reflection, however their effects were not visible in either the arterial pulse profiles or the impedance spectra of any animal. In the vasoconstricted state, the mean L/λ increased to 0.1219. This value, while not significantly different than the vasodilated ratio of 0.1187, again indicates the potential for discrete wave effects. In two animals, the arterial pulse contour showed some evidence of a small dicrotic wave and a small increase in the ΔPP. However, neither the individual impedance spectra or the mean spectra were significantly different than the control patterns.

It is evident that the L/λ ratio may be inappropriate as an independent indicator of wave transmission effects under many circumstances. This ratio, calculated under conditions of normal Rs, C, and f₀, may provide a rough estimation of the ideal location of the heart with respect to the peripheral reflecting site, but even then, it is subject to great variation. Figures 56 and 57 graphically illustrate that the L/λ ratio is primarily determined by peripheral vascular tone and not by heart rate. The use of this ratio may only be appropriate in the context of additional data such as the input impedance spectra and arterial pulse profiles.

**Wave Reflection in the Rat and Mouse**

Teleologically, cardiac energy expenditure in mammals is thought to be minimized by the correct matching of aortic length and the fundamental wavelength of the pressure pulse. In this manner, the
pulsatile pressure differentials required to drive blood flow are maximized in the peripheral vascular beds and minimized at the input to the arterial tree. This reduces the energy required to overcome the opposition to flow afforded by the systemic vasculature. Since arterial wall properties have been shown to be similar in mammals, the pressure pulse wavelength is defined by heart rate. As a consequence, the 1/4 λ concept described by Tubular models becomes a function of the matching of L and f₀. However, as mammals become smaller there is an increasingly disproportionate change in these two parameters hence, the beneficial correlation may be lost.

In this investigation, rats were shown to demonstrate discrete wave reflection effects under normal physiological conditions. Measurements of C, MAP, and Rs (normalized to body mass) were similar to larger mammals. The biomechanical tests demonstrated similar central vascular viscoelastic properties. The systemic impedance spectra indicated that although the minimization of impedance occurred at a higher frequency than in larger mammals, reflections contributed significantly to the reduction in cardiac energy expenditure. Arterial pulse profiles were typical of other larger mammals in that centrally, wave reflections summed with the incident wave after closure of the aortic valve thus minimizing peak systolic pressure and aiding diastolic coronary perfusion. Peripherally, pulsatile pressure was increased by peak systolic summation of the incident and reflected wave components thus increasing peripheral vascular perfusion. Calculations of the effective L predicted a major peripheral reflecting site
located at about the level of the iliac bifurcation. Under control conditions, the mean calculated $L/\lambda$ ratio was indicative of wave reflection effects however, the variation in individual ratios was quite large.

When increases in peripheral vascular resistance were produced, there were substantial alterations in all indicators of wave transmission properties in rats. The input impedance spectra showed evidence of both an increase in the magnitude of reflections and an earlier arrival at the heart. There was a significant increase in pulsatile pressures peripherally and a smaller increase centrally. The arterial pulse, recorded in both locations, was characterized by significant alterations in contour. However, contrary to observations of increased peripheral wave reflection in other measurements, the $L/\lambda$ ratio decreased as a consequence of the large increase in $C$ independent of any significant alteration in $f_0$.

The impedance spectra of vasodilated rats showed both a diminished magnitude and a delayed timing of wave reflections at the heart. Impedance amplitude in the lower frequency harmonics increased and there was a decline in the high frequency fluctuations in impedance moduli. Pulsatile pressures declined in both the central and peripheral waveforms and the pulse contours assumed an almost identical shape. The $L/\lambda$ ratio under these conditions was in conflict with the other indicators of wave reflection effects. The decline in $C$ and increase in $f_0$ decreased $\lambda$, thus $L/\lambda$ increased even though discrete wave reflection
effects were obviously diminished.

Indicators of cardiovascular performance and measurements of input impedance, arterial pulse profiles and $L/\lambda$ ratios in the mice in this study illustrated striking differences as compared with other mammals. In the anesthetized mouse, MAP was much lower, $C$ significantly slower, and $R_s$ much less, than other small mammals. Biomechanical tests indicated an increase in the distensibility of the central arterial vasculature supporting the observed decrease in the other variables. In both the control mean and individual input impedance spectra there were no minima or maxima of impedance and no phase crossover. Arterial pulse profiles demonstrated similar central and peripheral waveforms and a decline in peripheral pulsatile pressure as compared with the central value. The calculated $L/\lambda$ ratio in these mice, based on a theoretical first minimum of impedance of 15 Hz, was indicative of a lack of discrete wave reflection effects.

When the mice were subjected to large increases in peripheral vascular resistance, there was a substantial rise in both MAP and $f_0$. Under these conditions, there were slight alterations in the impedance spectra which were apparent as small high frequency fluctuations. However, in both the mean and in the individual spectra, there were no apparent minima or maxima and no phase crossover within the frequency range studied. The arterial pressure pulse contours showed evidence of peripheral peaking in two animals and a small diastolic wave centrally. While these observations suggest minimal wave reflection effects, their
influence on input impedance are inconsequential as a result of
the small magnitude of the reflections and the delayed return to
the heart. The $L/\lambda$ ratio increased slightly under these
conditions but did not strongly suggest the presence of discrete
wave reflection effects.

Significant declines in $R_s$ were met with large increases in
stroke volume in these mice however, heart rate was essentially
unaffected and mean arterial pressure remained unchanged. In this
treatment group, the impedance spectra were generally
characterized by a decline in the impedance magnitude of the
higher frequency components and again showed no minima or maxima
and no phase crossover at any frequency studied. The central and
peripheral arterial pulse profiles were nearly identical, but
there was a greater decline in the peripheral pulse pressure as
compared with the central value than was observed in the control
animals. The $L/\lambda$ ratio increased slightly, in contradiction with
the changes in the impedance spectra and pressure pulse
characteristics.

Conclusions
The initial premise of this investigation addressed the
correlation between aortic wavelength and body size as it related
to the minimization of cardiac energy expenditure in mammals.
Based on descriptions of similar anatomical and biomechanical
properties of the arterial vasculature, and an average mass-
independence of $C$, it was hypothesized that very small mammals
would be unable to maintain the correct matching of $L$ and
required to promote beneficial wave reflection effects. This investigation has provided strong evidence to support this hypothesis.

The results of this study indicate that, like larger mammals, rats can be characterized by Tubular models of hemodynamics. Under normal conditions, the interrelationships of L, f₀ and C are matched in these animals in a way that should minimize cardiac energy expenditure through the correct timing of wave reflections. Although the L / λ correlation appears to be at the lower limits of the 1/4 λ described by the Tubular theory, the benefits of L / f₀ matching appear to become more optimal as f₀ rises and/or Rs increases. There is an observed decline in systemic impedance as a result of these alterations. As such, cardiac efficiency may increase during periods of physiological stress or exercise in the rat.

While rats demonstrate discrete, beneficial, wave reflection effects, mice appear to prevent the detrimental cardiac effects of inappropriately timed wave reflections through adaptive physiological mechanisms. The increase in central aortic distensibility in mice, in combination with a lowered Rs, minimizes and distributes wave reflections so that their effects are diffuse. This appears to prevent summation effects at the heart which would increase peak systolic pressure and cardiac energy expenditure. Under conditions of extreme peripheral vasoconstriction the magnitude of reflections from the lower body appear to increase, and there may be some peripheral pressure
amplification, however, the elevated distensibility of the central vasculature still prevents their return to the heart. As a result, during stress or exercise mice may be able to increase peripheral perfusion without increasing systemic impedance.

Finally, the results of the induced alterations in peripheral vascular tone indicate that \( L / \lambda \) is determined by both \( C \) and \( f_0 \), and not by cardiac frequency alone. As such, the use of this indicator in isolation of other measurements is not adequate evidence of wave transmission effects.
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