The Low Frequency Dynamic Viscoelastic Properties of Human Aortic Valve Tissue

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SUMMARY Membranous samples of human aortic valve cusps were subjected to sinusoidal fluid pressure variations (frequency range, 0.5-5 Hz) to assess their dynamic viscoelastic properties. The storage (elastic) and loss (viscous) moduli and phase lag between the stressing function and response were found to be independent of the frequencies applied. The respective average values were 1.35 (SE = 0.06) x 10^6 dynes cm^-2, 4.14 (SE = 0.28) x 10^6 dynes cm^-2, and 0.033 (SE = 0.002) rad. The small phase lag indicates that the tissue would recover almost completely to its original state on removal of any applied stress, and this and the relatively low extensibility should be considered in the design of leaflet-type valve prostheses. The storage modulus of the aortic valve cusps when compared to that of the mitral leaflet shows the mitral leaflet to be almost twice as stiff as the aortic valve cusps. This finding led us to conclude that the vibrations of these two cardiac valves alone cannot contribute in any significant way to the production of the observed lower frequency of the first and the higher frequency of the second heart sounds and that other factors must be considered to explain this finding.

FOR OVER 10 years, cardiac valve replacement has been an accepted form of treatment for patients with diseased and incompetent valves but the ideal substitute for such valves has yet to be found. Valve substitutes can be classified broadly into mechanical prosthetic devices and tissue valves constructed from biological materials such as fascia lata and pericardium. Whereas mechanical prostheses suffer from the problem of thromboembolism, this complication is seldom observed with tissue valve implants. However, one major disadvantage with tissue valve substitutes is valve incompetence and eventual structural failure. This may in part be due to the unsuitability of the tissue substitutes in both elasticity and mechanical strength. The importance of knowing the mechanical properties of normal valve tissues has been stressed by many as a necessary prerequisite for finding a suitable substitute in the successful design of tissue valve prostheses.4,5

Yamada4 and Clark4 reported studies of the static elasticity of strips of human aortic valve cusps cut in different directions. Their results show qualitative similarity but are at variance quantitatively. Yamada used a Schopper tension tester and reported a stress value of 6.5 x 10^6 dynes cm^-2 at 3% strain for strips cut in the circumferential direction and 3.0 x 10^6 dynes cm^-2 for strips cut in the radial direction, while Clark, who employed an Instron machine, reported corresponding values of 5.6 x 10^6 dynes cm^-2 and 2.6 x 10^6 dynes cm^-2, respectively, at the same level of strain. Mundth et al.4 also reported pressure-volume studies for membranous samples of canine aortic valve tissue and Wright and Ng6 performed similar studies on human aortic valve cusps.

Since the aortic valve is in a dynamic state of stress in vivo, we investigated the dynamic elasticity of human aortic valve cusps for low stressing frequencies. Large disk-shaped samples of the membranous valve cusps were used rather than strips of tissue, as we believed that, because of the arrangement of connective fibers in the tissue, cut edges might weaken the tissue and hence not provide true information on its mechanical properties.

Methods

THEORY

When a linear viscoelastic material is subjected to a sinusoidal mechanical stress (σ), deformation is resisted by both an elastic restoring force and a viscous resistance. In the steady state condition the resulting strain (ε) also is sinusoidal but will be out of phase with the stress, as shown in Figure 1. In view of this phase shift φ (radians), the stress therefore can be resolved vectorially into a component in phase with the strain and a component 90° out of phase with the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain. These stress components when divided by the strain.
phase) of membranous samples of human aortic valve cusps. An acrylic plexiglass cylinder 1 cm. in internal diameter was used. There was a circular opening 1 cm in diameter at one end and a piston at the other. The piston was operated by an a.c-d.c Universal gear motor (Bodine type NSE-11R, model 108) connected to a frequency control built in our laboratory. Connected between the motor and the piston was an electromagnetic clutch with a spring brake (Precision Instruments DX-9) and a microswitch.

Two annular plates made of acrylic plexiglass were mounted on an opening (1 cm in diameter) of the cylinder and served as clamps. The internal diameters of these clamps were 1 cm. They were constructed with a groove around the orifice of the lower support, 1 mm away from the edge. At this same position, the upper removable clamp had a matching raised circular ring so that the clamps fitted closely when a piece of material was placed between them. The clamps held the material firmly and thus prevented slippage.

The cylinder was filled completely with 0.9% saline and, when pressure was applied by moving the piston inward, material mounted on the clamps was stretched and bulged upward. The applied pressure was measured with a transducer, with negligible delay in response (Statham P23DEc), connected to the side of the cylinder, and the height of the bulge was measured by a light weight core (0.52 g) linear variable differential transformer (LVDT, Schaevitz, type 100 MHR) which, according to the manufacturer's specifi-
cation, offered negligible friction during use. Signals from both transducers were connected to the amplifier assembly of a Beckman Dynograph recorder (type RM) and the amplified signals were displayed on a storage oscilloscope (Tektronix 5103N). When the piston moved in a sinusoidal fashion, pressure variations were transmitted down the cylinder and these caused sinusoidal bulging of the material held in the clamps. Thus, the oscilloscope displayed two sinusoidal traces: pressure (P) and bulge height (h) of the same frequency. Any phase shift (φ) between the applied sinusoidal stress and the resulting sinusoidal strain was displayed as a phase shift between the pressure and bulge height tracings on the oscilloscope.

Aortic valves that appeared normal at autopsy were obtained from the hearts of six female and eight male subjects, 40–76 years old at the time of death, who had had no history of aortic valve disease. The cusps were removed, identified according to site of attachment to the aorta, and kept in 0.9% saline-0.01% Merthiolate solution at a temperature of 6°C until tested. This antibacterial solution preserved the mechanical properties of collagen and elastin.7 The valve cusps all were removed not more than 12 hours after death, and, as far as possible, all three cusps from each valve were tested. Pieces greater than 1 cm in diameter were cut from the central portion of each cusp, where the thickness was found to be fairly uniform. This tissue then was placed over the opening of the saline-filled cylinder and clamped. During the filling of the cylinder and the fastening of tissues, care was taken to ensure that no air bubbles were trapped. As the aortic surface of the cusp is the surface on which most pressure is applied in vivo, the tissues were mounted with this surface down. The mounted tissue then was subjected to sinusoidal pressure variations and the applied pressure (P) and the resultant bulge height (h) were recorded simultaneously when a steady state had been achieved. Photographs were taken of these traces, and from them values of stress and strain were calculated. To increase the accuracy in the determination of the phase shift, traces at sweep rates as high as 63.5 cm/sec, and with the peak regions enlarged, also were recorded. Each membrane sample was subjected to sinusoidal stresses at five different frequencies ranging from 0.5 Hz to 5 Hz and all the experiments were performed as soon as possible at a room temperature of 22 ± 1°C and not more than 3 days after removal of the valve from the heart. To obtain stress values from the measured pressure values, measurement of the average thickness of the nearly uniform sample was required, and this was made with a microscope.

ANALYSIS

Since σ0, the maximum stress, and ε0, the maximum strain, were required they were calculated from the measured values for pressure (P) and bulge height (h). For a segment of a sphere cut by a single plane of radius (r), the area (A) of the convex surface is given by

\[ A = \pi (r^2 + h^2) \]  

where h is the height of the cut segment of the sphere. For a thin piece of tissue stretched to form part of a sphere from a plane configuration, h would represent the height of the bulge and r the radius of the original circular unstretched tissue. Hence, the initial surface area of the unstretched tissue was

\[ A_0 = \pi r^2. \]  

Therefore, the increase in area was

\[ \Delta A = A - A_0 = \pi h^2. \]  

Hence the areal strain

\[ \epsilon = \frac{\Delta A}{A_0} = \frac{h^2}{r^2}. \]  

Therefore ε0, the maximum strain, could be calculated from Equation 7 when the bulge height h was maximum.

If we neglect the small edge effects and the low bending stresses, the law of Laplace can be applied to deduce the stresses on the tissue. For a membrane forming part of a sphere the law of Laplace states that

\[ P = \frac{2T}{R} \]  

where P is the pressure difference across the membrane, T the tension per unit of length on the membrane, and R the radius of curvature of the spherical surface. Simple calculations show that R is related to the bulge height h by

\[ R = \frac{r^2 + h^2}{2h} \]  

hence

\[ T = \frac{P(r^2 + h^2)}{4h}. \]  

In our study P was the measured gauge pressure and, if d was the thickness of the membrane, then the membrane stress is

\[ \sigma = \frac{T}{d} = \frac{P(r^2 + h^2)}{4hd}. \]  

The maximum stress, σ0, was calculated from Equation 11, when P was maximum. The value of r in our study was 0.5 cm.

From the maximum values for P and h for each trace, and knowing r and d, σ0 and ε0, the storage modulus E' and the loss modulus E'' could be calculated for each frequency.

In the above analysis several assumptions were made, namely, that the tissue was stretched to form part of a sphere, that the bending stresses and edge effects due to clamping of the specimen were negligible, and that the spherical cap formed was thin-walled. These assumptions must be justified.

To show that the tissue bulged to form a spherical cap, the elevations of different regions of the cap were measured.
From the maximum height of the cap the expected elevations of these regions were calculated by assuming a spherical bulge. Most of the calculated and measured values were in relatively good agreement, differing on the average by 15.8% (SE = 1.9%, range = 0-30%). Hence, to a first approximation our assumption of a spherical configuration is justified.

In engineering practice it is customary to consider a surface as thin-walled if the $R/d$ ratio is approximately 10 or larger. In our experiments the bulge height was of the order of 0.1 cm, giving an $R$ value of about 1.3 cm. Since $d$ was in the order of 0.06 cm the $R/d$ ratio was about 22. Hence, the spherical cap can be considered as thin-walled.

In the theory of plates and shells the bending rigidity of a thin isotropic plate or a thin shell is proportional to $d^4$, whereas the extensional rigidity is proportional to $d$, the thickness. For values of $d$ less than 1, $d^4$ would be smaller than $d$. Since the thickness of our specimens was of the order of 0.06 cm, the bending stresses would be small in comparison to the extensional stresses.

To show that the edge effects were negligible, experiments were performed with a piece of rubber using two different openings of the cylinder (0.6 cm and 1.0 cm in diameter). At the same strain, the results obtained with rubber were comparable, differing by less than 5%, and thus suggested that the edge effects can be ignored.

**Results**

The upper trace of Figure 3A shows a typical oscilloscope trace of the imposed sinusoidal pressure; the lower trace shows the resulting sinusoidal bulging of the tissue. To visualize any possible phase shift with greater accuracy, the peaks of the two traces were enlarged and the sweep rate was increased. Figure 3B shows such an enlarged tracing (top trace for pressure, lower trace for bulge height), and the response was observed to lag behind the forcing function. Since these traces were good approximations of sine waves, the use of Equations 2 and 3 for the evaluation of $E'$ and $E''$ was justified. Because these traces were recorded as changes with time on the oscilloscope the phase shift, $\phi$, in radians, was calculated from the following formula

$$\phi = 2\pi f \Delta t,$$

where $f$ is the imposed frequency in Hertz and $\Delta t$ in seconds was the time difference between the occurrence of the two peaks.

The results shown in Figures 4 and 5 cover a small range of areal strain of 2-4.5%. Statistical analysis showed that the response of tissues tested immediately after removal from the heart was not significantly different from that of tissues stored for up to 3 days. The variation of phase lag, $\phi$, with frequency is shown in Figure 4. All values were found to be low and there appeared to be a drop in $\phi$ at 5 Hz. However, a simple analysis of variance indicated that the differences between the values for $\phi$ were not significant ($P > 0.05$) and an average value of 0.033 (SE = 0.002) rad was found. The low values for $\phi$ indicate that the tissue has little viscous resistance, hence it would recover almost completely to its original state on removal of any applied stress.

**Figure 3** A typical response trace taken from the storage oscilloscope. A: the top trace shows the imposed sinusoidal pressure; the lower trace, the resulting sinusoidal bulging of a clamped specimen. B: peak of the pressure and bulge height traces enlarged and the sweep rate increased.

This property is advantageous to a structure that is under constant dynamic stress.

Figure 5 shows the variations of the dynamic modulus with frequency. The data points on the top of the graph are for the storage modulus, $E'$, and those at the bottom for the loss modulus, $E''$. The slope of the best straight line through the $E'$ values was found to be not significantly different from zero ($P > 0.3$). Thus again $E'$ was quite independent of the frequency values investigated, and an average value of 1.35 (SE = 0.06) $\times 10^4$ dynes cm$^{-2}$ was found. As both $E'$ and $\phi$ were found to be independent of frequency, $E''$ would be expected to behave in a similar manner. An average value of 4.14 (SE = 0.28) $\times 10^4$ dynes cm$^{-2}$ was found.

The dynamic elastic moduli and phase lag did not vary
worth considering in the design and use of either synthetic or aortic valve cusps are relatively inextensible and that the leaflets are almost twice as stiff as the aortic valve cusps. This low extensibility of the valve cusps agrees well with results of the studies by Swanson and Clark, who used silicone rubber valve casts. They reported that the leaflet length of the valve varies negligibly with applied pressure.

In the explanation of the origin of the first and second heart sounds, some authors are of the opinion that these sounds originate from the vibrations of the atrioventricular and semilunar valves on closure. More recent views suggest that these sounds are produced by the accelerations and decelerations of blood which give rise to vibrations of the heart walls and the major blood vessels. Guyton suggested that the observed differences in the frequencies of the first and second heart sounds could, in part, be due to the differences in the elastic moduli of the atrioventricular and semilunar valves. He suggested that the lower frequency of the first sound could be due to the lower elastic modulus of the atrioventricular valves and the walls of the ventricles compared to the elastic modulus of the semilunar valves and the arterial walls. He appears to have simplified the problem, which if analyzed in detail will undoubtedly be very complex. However, if we assume that the loading and configuration of both the mitral and aortic valves after closure do not differ significantly, then the remaining major factor governing the vibrational frequency of the valves will be the elastic moduli of the valve tissue itself. The results presented here and those reported in a previous study on the mitral valve show that the elastic modulus of the mitral leaflets is greater than that of the aortic.

The results presented in Figures 4 and 5 show that human aortic valve cusps are relatively inextensible and that the material has a very low loss value. These characteristics are worth considering in the design and use of either synthetic or biological materials in leaflet-type valve prostheses. This low extensibility of the valve cusps agrees well with results of the studies by Swanson and Clark, who used silicone rubber valve casts. They reported that the leaflet length of the valve varies negligibly with applied pressure.

FIGURE 5 The top graph shows the variation of the storage modulus (E'<SUP>′</SUP>) with frequency; the lower graph shows the variation of the loss modulus (E'<SUP>″</SUP>) with frequency. The vertical bars indicate the standard error.

significant between the three cusps of the aortic valve. This finding is in agreement with results of the pressure-volume studies by Wright and Ng. The results also were similar for both sexes and did not appear to depend on age in our group of specimens from subjects in the 40- to 76-year age range.

Discussion

The experimental approach we adopted to study the viscoelastic property of human aortic valve cusps was designed to simulate the in vivo condition under which the aortic valve is stressed. This was achieved by the application of sinusoidal fluid pressures on the aortic surface of the aortic valve cusps. This investigation differs from others in that dynamic rather than static tissue responses were studied and membrane samples instead of tissue strips were employed. The frequency range was chosen to include the spectrum of frequencies to which a normal functioning valve might be subjected.

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Redistribution of Collateral Blood Flow from Necrotic to Surviving Myocardium following Coronary Occlusion in the Dog

HEINZ O. HIRZEL, M.D., GEORGE R. NELSON, M.D., EDMUND H. SONNENBLICK, M.D., AND EDWARD S. KIRK, PH.D.

SUMMARY Early changes in collateral blood flow after acute coronary occlusion may be critical for survival of ischemic myocardium. We used 15-μm radioactive microspheres to study myocardial blood flow in thoracotomized dogs 10 minutes and 24 hours after occlusion of the left anterior descending coronary artery (LAD). The ischemic area was delineated by dye injected into the distal artery, and identification of potentially ischemic samples was confirmed by a newly developed technique in which microspheres were excluded from the normally perfused LAD. Layers were separated into necrotic or newly developed regions. In combination with a developing collateral supply this process may be essential for sparing myocardium after coronary occlusion.

SURVIVAL of myocardium after coronary occlusion depends on a balance between supply and demand for oxygen. Measures which reduce myocardial necrosis by reducing oxygen demand must be coupled with an eventual increase in oxygen supply if function is to be restored to the ischemic myocardium. Thus, ultimate salvage of ischemic myocardium should depend on the development of an adequate coronary collateral supply. Numerous studies on animals have demonstrated the eventual development of coronary collaterals following gradual12 or abrupt coronary occlusions,12 but the changes in collateral blood flow in the early critical period after occlusion remain poorly understood.

Several studies13-17 demonstrated no evidence for an increase in coronary collateral blood flow for several days after an acute coronary occlusion but others reported increases within 24 hours in peripheral coronary pressure, xenon clearance, and retrograde flow,4 or in myocardial flow measured with microspheres.9-11 However, even with rapid development of collaterals, necrosis or a "no reflow" phenomenon12 may alter the distribution of flow within the myocardium by the time sufficient flow is available to the ischemic myocardium. The present study was undertaken to examine in detail the changes in myocardial blood flow that occur in ischemic myocardium within 24 hours after an acute coronary occlusion.

**Methods**

**EXPERIMENTAL PREPARATION**

Twenty mongrel dogs weighing 11-22 kg (average, 15.2 kg) were anesthetized with sodium pentobarbital (27.5 mg/kg, iv). Additional pentobarbital was administered as...
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