In vivo cinematographic analysis of behavior of the aortic valve

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Van Steenhoven, A. A., C. W. J. Verlaan, P. C. Veenstra, and R. S. Reneman. In vivo cinematographic analysis of behavior of the aortic valve. Am. J. Physiol. 240 (Heart Circ. Physiol. 9): H286-H292, 1981.—In open-chest dogs direct-cinematographic high-speed recordings of aortic valve movement were made using a thin flexible fibroscope. Simultaneously ECG, ascending aortic flow (electromagnetically), and the pressures in the aorta, left ventricle, and left atrium were recorded. Replacement of blood by a transparent liquid (Tyrode solution) was done with two roller pumps, one connected to the left atrium and the other to the femoral artery. Free outflow occurred through a cannula in the pulmonary artery. Comparison of the film frames with the aortic flow signals revealed that 1) the valve was completely open at the moment that aortic flow had reached about 75% of its maximum value; 2) the opening time was 32 ms; 3) valve closure started before the onset of aortic flow deceleration; 4) at least 80% of the closure was completed before aortic flow becomes zero; 5) complete valve closing coincided with the moment of maximum backflow in the valve; 6) the shape of the valvular orifice at complete opening was almost circular; and 7) fluid viscosity had no significant effect on valve closure.

Aortic valve opening; valve closure; aortic flow; model study

Aortic Valve Function has attracted scientific interest since Leonardo da Vinci made his first reports in 1513 (7). Direct vision of the aortic valve leaflets was first achieved in vitro, using a pulse duplicator (3, 4, 8, 10). However, the valvular movements observed in these studies in nonbeating hearts may not be representative of the actively contracting heart (13).

Observation of aortic valve action in the beating heart can be done both indirectly and directly. Indirect methods include X-ray techniques, using either contrast material (5, 11, 19, 20) or radiopaque markers (21), as well as echocardiography (9). A major disadvantage of these methods is that either the time resolution is small or the movement of only two of the three leaflets can be observed. Direct observation of aortic valve action in the intact animal can be achieved with cinematographic techniques. The main problem with this method is the replacement of blood by a transparent medium for a period of time sufficiently long to allow observation and photographic recording of the valve. The duration of direct recording of the aortic valve leaflets under physiological circumstances is limited because of the small oxygen content of the hemoglobin-free perfusion fluid (Tyrode solution). Using this direct cinematographic technique Hider et al. (6) were able to study aortic valve movements as seen from the ventricular apex at a film speed of 64-80 frames/s. By perfusing the coronary arteries separately with blood the observation period could be prolonged (2, 13, 14). However, the film speed had to be reduced to a value less than 64 frames/s due to the presence of some blood in the left ventricular cavity.

In the present study, aortic valve motion as seen from the aorta was studied in open-chest dogs by use of direct high-speed cinematography. The motion of the valve during the cardiac cycle was compared with the instantaneous ascending aortic flow tracing. The results thus obtained were compared with those obtained in a theoretical model (17). A preliminary abstract of the animal study has been published (16).

METHODS AND MATERIALS

Experimental procedure. Experiments were performed on 15 mongrel dogs of either sex, unknown age, and ranging in weight from 25 to 45 kg. The animals were premedicated with Hypnorm (1 ml/kg body wt im; 1 ml Hypnorm contains 10 mg fluanison and 0.2 mg fentanyl base). Anesthesia was induced with pentobarbitonal sodium (10 mg/kg body wt iv) and, after endotracheal intubation, was maintained with oxygen-nitrous oxide. Ventilation was kept constant during the experiment with a positive pressure respirator (Bird).

The ECG was derived from limb leads. The chest was opened through the left fifth intercostal space and the heart was suspended in a pericardial cradle. Millar catheter-tip micromanometers (PC 470) were used to measure aortic and left ventricular pressure. Aortic blood flow was measured with an electromagnetic flow probe, mounted on the ascending aorta. The probe was connected to a sine-wave electromagnetic flowmeter with a carrier frequency of 600 Hz and an upper frequency response of 100 Hz, −3 dB (Transflow 600). End-diastolic flow in the aorta was used as zero-reference. The flow probes were calibrated in vitro, previous to the experiments. Qualitatively no significant influence of the fibroscope (see below) on the flow curve could be detected, which indicates that the accuracy of the flow measurement is hardly affected by the presence of the scope. The variables to be measured were recorded on a multichannel physiolog-
critical recorder (Schwarzer) and on an electromagnetic tape recorder (Ampex PR 2230). The upper frequency response of the recording system, which is limited by the physiological recorder, was 280 Hz, -3 dB.

For direct cinematographic recording of aortic valve movement, under fluoroscopic control, a thin (4 mm) flexible fibroscope (Olympus BF 4C2) was placed in front of the valve through the left carotid artery. In water the optical system has an angle of vision of 45°. Light from a mercury vapor lamp (American Cystoscope Makers FCB 1000) was emitted from the tip of the lens system at an intensity of 400,000 lux and a color temperature of 5,000°K. Aortic valve motion was filmed with a high-speed film camera (Hitachi-Hilmac) at a speed of 200 frames/s using Kodak video news film 7240 (125 ASA). By special processing procedures a speed of 1,000 ASA was reached. A low-light video camera (Philips BM 8024) was placed at the ocular of the film camera, to obtain a direct view of the valve on a video monitor (Philips EL 8125). The coupling of optical and electrical signals was achieved using a 50-Hz timing signal on both film and tape recorder.

After the devices to measure the various variables were placed in position, the pulmonary veins were ligated and the blood was replaced by a transparent Tyrode solution, either with (3.3 g/100 ml) or without gelatine (Union Chemique Belge pronanalyse). The fluid had a temperature of 37°C and was saturated with a gas mixture consisting of 5% CO2:95% O2. Perfusion was performed by two roller pumps (Sarns 6013), one connected to the left atrium and the other to the femoral artery. This second pump was essential to maintain peripheral arterial blood pressure at physiological levels. Free outflow occurred through a cannula in the pulmonary artery.

After the experiment the heart was removed, and the aortic radius as well as the distance of the flow probe to the center of the valve were measured under unloaded conditions.

Data processing. A sequence of film frames of five successive heart beats was chosen for analysis. An additional condition for the choice was a reasonable degree of uniformity of the instantaneous aortic flow and the aortic and left ventricular pressure curves during these five beats. These frames were analyzed with an analyzing projector (Analector, Oude Delft), and drawings were made of the instantaneous cusp positions. From the drawings the instantaneous area of valve opening was measured with a planimeter (Ott 31). For proper evaluation of the valvular behavior, the orifice area of the aortic valve was calculated as an instantaneous function of time and compared with the instantaneous flow within the valve. The measured aortic flow signal was shifted over about 8 ms because of the position of the flow probe with respect to the valve and the electronic delay in the flowmeter system (~1.5 ms). The time shift due to the position of the probe was calculated from the measured distance between flow probe and valve, assuming a pulse-wave velocity of 4 m/s (12).

Coupling between optical and electrical signals may be subject to two distinct errors. First, all the film records were made at a camera speed of about 200 frames/s. This restricts the accuracy of the time coupling to about 3 ms. Second, at a distance between probe and valve of about 30 mm, variations of 15% in the pulse-wave velocity result in a time error of about 1 ms.

The valvular behavior as well as the corresponding aortic flow signals were averaged over five successive heart beats by normalizing these variables to their maximum value. The time scale (width of flow curve) was normalized by relating time to the time interval between 25% of the peak flow during acceleration and 5% of the peak flow at the end of deceleration, as explained in Fig. 1.

The reason for choosing these particular scaling factors was twofold. First, in the averaging procedure the moments of opening and closing of the valve must be defined reliably. Because it is most probable that the valve opens at the beginning of the flow curve and closes at the end of it, the normalization of time in relation to the time span between these two events is reasonable. Second, the steeper the course of the flow curve the more accurate the unit of time, relevant for the normalization of time, can be determined. Obviously the single flow curves thus obtained show the same amplitude and the same width. After having thus established the basic definitions for analysis of measurements, the instantaneous flow curve following each normalized time step, being 1/50 of the normalized time unit, was averaged over five heart beats. This finally resulted in an average normalized instantaneous flow curve. Evidently, the curve of valve orifice area fraction (a), defined as the ratio of the instantaneous and maximum orifice area, as obtained from the film frames, was also of a normalized nature with respect to time, because of the coupling to flow. However, due to some inaccuracy in the determination of the coupling, there may be a systematic time error in each separate measurement. These errors were estimated from the time difference between the various curves at a = 0.50, both during acceleration and deceleration. A relative shift of the curves over the time difference thus found compensates for the error. The valve orifice area fraction values presented are the averaged ones at each time step, similar to the procedure followed for the aortic flow. Finally both the normalized average flow and the normalized average curve of valve orifice area fraction were restored to physical quantities, i.e., real flow and real time, using the mean values of the five heart beats. This resulted in a curve of mean valvular behavior in relation to a curve of mean instantaneous aortic flow.

Figure 1 shows five successive single instantaneous flow and valve orifice area fraction curves, after normalization of amplitude and time. It clearly shows both the similarity of the flow signals and the large variations in the maximum value of the valve orifice area fraction. The level at which the normalization of the flow curve with respect to time was performed, was chosen rather arbitrarily. Therefore, we repeated the procedure also on the basis of the time delay between the 50% levels of peak flow during acceleration and deceleration, instead of between the original 25 and 5% levels. We did not find relevant differences between the curves thus determined.

The analysis of the film frames was hampered by the fact that sometimes, especially when a fully opened valve was filmed, the valve orifice was not completely visible.
RESULTS

Generally, the time between the start of perfusion and the beginning of filming aortic valve movements was less than 1 min. A regular heart rhythm and relatively normal aortic flow, as well as aortic and left ventricular pressure values were maintained during the filming period of about 2 min in all experiments. In nine experiments the film images were of a quality sufficient for reliable analysis. In these experiments 12 measurements were performed.

Heart rate generally decreased during perfusion. In the experiment shown in Fig. 3 the viscosity of the perfusion fluid was equal to that of blood ($\eta = 3 \times 10^{-3}$ N·s/m²). The corresponding mean curves for instantaneous aortic flow and valvular behavior are shown in Fig. 4. Similar results were obtained at low viscosity ($\eta = 10^{-3}$ N·s/m²), as shown in Fig. 5.

In such a case the analysis was performed by using the visible part between two commissures and the center of the aorta. To test the applicability of this method, in one case where the valve was completely visible throughout systole, the instantaneous valve orifice area fraction was determined from one-half as well as one-third of the valve orifice. The comparison with the values determined from the complete valve orifice is shown in Fig. 2. No relevant difference was found between the results throughout systole. To check the reliability of the calculation of the mean curves, we determined the 95% confidence level of the mean derived from five successive measurements averaged over all time steps. For the flow values this resulted in a mean confidence level of 3.5% of peak flow; whereas for the valve orifice area fraction this value was about ±0.06.

FIG. 1. Typical aortic flow (AF) and valve orifice area fraction (a) curves for 5 successive beats after normalizing amplitude and time. Zero time coincides with 25% maximum flow. See text for discussion.
The experimental results for a low and high viscosity (equal to that of blood) averaged over the experiments with a comparable heart rate are shown in Table 1. These data show that 1) the valve was completely open at the moment that aortic flow had reached about 75% of its maximum value (relative opening flow), the opening time was about 32 ms; 2) the valve had already closed for some 8% at the onset of deceleration of aortic flow (midsystolic valve orifice area fraction); 3) at the moment that the decelerating flow became zero, the major part (78-80%) of valve closure was already completed (end-systolic valve orifice area fraction); 4) the time difference between complete valve closure and the moment of maximum back flow in the valve (closing time delay) was negligible, indicating that both events virtually coincide; and 5) the differences in the values corresponding with low and high viscosity were not statistically significant.

From the present experiments it is not easy to determine the proper shape of the orifice, since during midsystole often only part of the completely opened valve could be seen. Moreover, the edges of the leaflets were rather vague. Therefore only in a few experiments the real geometry of the leaflets could be described in a well-defined way. The shape of the valve orifice as a function of time under physiological conditions (expt shown in Fig. 5) is drawn in Fig. 6. From this it may be concluded that at the onset of systole the valve leaflets moved rapidly until the valve had opened completely. The shape of the orifice at complete opening was virtually circular. This geometry was maintained during the major part of systole, although according to the Figs. 5 and 6 the surface area of the orifice gradually decreased during this phase of the cardiac cycle. Near the end of systole the shape of the orifice gradually changed into a triangle and finally the leaflets closed completely. When the valve was open, generally some fluttering of the leaflets was observed at a frequency of 50-100 Hz. The observed total deflection was small (about 0.6 mm) and no significant difference could be detected between the conditions of either high or low viscosity.
DISCUSSION

Animal experiments. In this study the behavior of the aortic valve was investigated using a modification of the cinematographic technique as originally described by Hider et al. (6). The major improvement made was the use of the two roller pumps to maintain peripheral arterial blood pressure at physiological levels. After the start of perfusion about 5 min are available for direct cinematographic high speed recording of the aortic valve movement. The present study indicates that during systole, valve behavior in relation to aortic flow can be described as follows. The opening of the aortic valve at the onset of systole proceeds very fast. Valve opening was completed within approximately 32 ms. Aortic flow has then reached a value of about 75% of its maximum value. The shape of the valvular orifice at complete opening is practically circular. Valve closure already starts during the acceleration phase of the aortic fluid. At the onset of the deceleration phase about 8% of the closure is accomplished. The gradual closure continues during the first part of fluid deceleration. Near the end of systole the valve closes swiftly. At the moment of zero aortic flow, approximately 80% of the closure is completed. The remaining 20% of closure is accomplished during the phase of back flow in the instantaneous aortic flow tracing. The findings suggest that complete aortic valve closure coincides with the moment of maximum backflow in the valve.

The relative opening flow found in the present study is high as compared to the relative opening flow of 40% as reported by Laniaido et al. (9). In their study, however, no reference is made to correction for the position of the flow probe with respect to the aortic valve. Neglecting this (estimated delay 7 ms) can readily underestimate the relative opening flow by some 20% (Fig. 4). Furthermore, echocardiography is probably not as precise as cinematography. The valve opening time of 32 ms as found in our study is significantly more than described in literature (ranging between 9 and 24 ms) by such investigators as Hider et al. (6), Mercer (11), Laniaido et al. (9) and Thubrikar et al. (21). The reason for this may be that the film speed used in our study is higher than that used by Hider et al. (6), which together with the direct method of observation allows a more precise determination of the onset of valve opening. On the other hand, the valve opening time found in our study might be too long due to a relatively slow acceleration of instantaneous aortic flow under the experimental circumstances.

The practically circular shape of the valvular orifice at complete valve opening, as found in the present study, is in agreement with the findings of Hider et al. (6), Padula

![FIG. 4. Typical relationship between aortic flow (AF) and aortic valve behavior (a) at high viscosity (equal to blood). Solid lines, experimental results. Dotted line, systolic valve closure as determined in theoretical model (17). Zero time corresponds to onset of flow deceleration.](image)

![FIG. 5. Typical relationship between aortic flow (AF) and aortic valve behavior (a) at low viscosity. Solid lines, experimental results. Dotted lines, systolic valve closure as determined in theoretical model (17). Zero time corresponds to onset of flow deceleration.](image)

### Table 1. Aortic valve behavior at high (equal to blood) and low viscosity of perfusion liquid

<table>
<thead>
<tr>
<th>Parameters</th>
<th>High Viscosity (η = 3 x 10^-3 N·s/m²)</th>
<th>Low Viscosity (η = 10^-3 N·s/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opening time, ms</td>
<td>33.3 ± 6.4</td>
<td>30.0 ± 5.6</td>
</tr>
<tr>
<td>Relative opening flow</td>
<td>0.76 ± 0.12</td>
<td>0.72 ± 0.16</td>
</tr>
<tr>
<td>Mid-systolic valve orifice area fraction</td>
<td>0.91 ± 0.03</td>
<td>0.92 ± 0.08</td>
</tr>
<tr>
<td>End-systolic valve orifice area fraction</td>
<td>0.14 ± 0.04</td>
<td>0.22 ± 0.17</td>
</tr>
<tr>
<td>Closing time delay, ms</td>
<td>0.4 ± 0.1</td>
<td>1.5 ± 1.7</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>69 ± 11</td>
<td>74 ± 14</td>
</tr>
<tr>
<td>Measurements</td>
<td>(n = 4)</td>
<td>(n = 5)</td>
</tr>
<tr>
<td>Animals</td>
<td>(n = 4)</td>
<td>(n = 4)</td>
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</table>

Values are means ± SD based on the number of measurements shown; however, each measurement is the average of five cycles as discussed in the text.
The finding that valve closure already starts during the acceleration phase of aortic flow confirms the qualitative findings of Laniado et al. (9) and Thubrikar et al. (21). The mechanism of this early start of closure is incompletely understood.

The observed valve closure of 80% during flow deceleration agrees with the valvular behavior as observed in model experiments reported by Bellhouse and Talbot (1). Laniado et al. (9), however, found that final closure is achieved at the moment that the decelerating flow in the ascending aorta becomes zero. The difference between their results and ours may be explained from their neglecting of the time shift due to the position of the flow probe on the ascending aorta.

The present experiments indicate that the viscosity has no significant influence on valve closure. The finding of Hider et al. (6) that the fluid viscosity seems to affect the shape of the maximum valve orifice could not be confirmed, no more than their statement that the leaflets

FIG. 6. Photographs (A) and drawings (B) of shape of valve orifice as function of time.
of the aortic valve are stable at low as well as at high viscosity. In our experiments, high-frequency fluttering could be observed during mid systole in both conditions mentioned.

Theoretical prediction of aortic valve closure. An important aim of the present experimental animal study was to test the validity of a theoretical model of aortic valve closure (15). This model is based upon the observations made of the closing behavior in a two-dimensional analogue of the aortic valve during deceleration of the mainstream. The conditions simulate the situation in the human aorta (17). In the analogue the following observations were made during flow deceleration: 1) the cusp rotates slowly into the aorta around its point of attachment without changing its shape; 2) the velocity profile of the mainstream under the cusp remains virtually flat; and 3) in the aorta at the rear of the cusp a region of recirculation is formed. We also showed that the vortex trapped within the sinus of Valsalva did not affect valve closure essentially (18), and we suggest that the function of this cavity is to keep the pressure differences small at the sinus side of the cusp. This is supported by the experimental finding by means of the analogue, that the presence of only a small cavity is essential for the mechanism of systolic valve closure (17).

From these experimental results a simplified theoretical model was designed (17), assuming that the pressure on the sinus side of the leaflet is constant and equal to the pressure underneath the free edge of the cusp. Two additional assumptions were made: the leaflet stays straight, and the mean pressure difference across the leaflet is equal to zero because of its negligible mass. For the three-dimensional model the cusps are assumed to have a truncated cone shape, whereas the aorta is considered to have a rigid tube shape. This model describes valve closure to be due to the adverse pressure gradient during flow deceleration. An equation was obtained that relates the aortic fluid velocity within the valve to the displacement of the leaflets. Note that the theory only holds for valve closure during flow-deceleration phase.

The theoretical curves for systolic valve closure, as shown in Figs. 4 and 5, are calculated from the data for instantaneous flow and valve geometry. The curves thus obtained agree reasonably with the results of the animal experiments, which suggests that the model describes the mechanism of valve closure during flow deceleration fairly well. However, the differences between theory and animal experiments in the two experiments mentioned are of different natures. This might be due to the simplifications made in the model for the cusp shape and disregard of the geometrical distortion caused by pressure changes within the valve orifice during the cardiac cycle. In the experiment shown in Fig. 4, for example, the systolic aortic pressure drop was significantly lower than that in the experiment represented in Fig. 5 (5 vs. 8 kPa).

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REFERENCES