

# An In-Vitro Technique for Assessment of Thrombogenicity in Mechanical Prosthetic Cardiac Valves: Evaluation with a Range of Valve Types

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**Background and aim of the study:** An in-vitro technique has been developed to assess the flow-induced thrombosis of artificial heart valves, using renneted milk as a blood analog. Previous studies have demonstrated similarities between the clotting of blood and milk on both microscopic and macroscopic scales. The study aim was to further validate the milk test by comparing the locations of milk clot to those of thrombus formation on a wide selection of mechanical heart valves.

**Methods:** Nine different valves were tested in the aortic position of a model heart chamber in the Edinburgh milk rig. These included caged-ball valves (Starr-Edwards silastic ball with bare struts and metal ball with cloth-covered struts), tilting-disc valves (Björk-Shiley Standard, Björk-Shiley Monostrut, Medtronic-Hall, and Ultracor), and bileaflet valves (St. Jude Medical, Edwards-Duromedics, and CarboMedics). Renneted milk was

pumped through the valves for 30 min at 2 l/min, 70 bpm pulsatile flow. After each run, valves were photographed for comparison with documented sites of thrombosis.

**Results:** All valves developed milk clot in specific, reproducible locations when run in the aortic position. Milk clot was found on the struts of caged-ball valves and tilting-disc valves, and around the hinge mechanism of the bileaflet valves. This compared favorably to documented cases of thrombosis in vivo.

**Conclusion:** Renneted milk may be used to model flow-induced thrombus formation and to predict the thrombogenic sites of mechanical heart valves. Whilst it is not suggested that milk mimics the entire blood coagulation cascade, these results indicate that such behavior may not be necessary for predicting fluid mechanically induced clotting.

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The recent failure of animal trials to detect thrombosis in the Medtronic Parallel valve (1) has exposed the existing need for a reliable method to assess the thrombogenic potential of heart valves prior to clinical testing. Researchers at Edinburgh University have developed a technique using renneted milk for the detection of potential clotting sites. The likenesses between milk clotting and blood clotting have long been noted (2), and previous studies have demonstrated similarities between the mechanisms of clotting on both a microscopic and macroscopic scale (3).

In replicas of Petschek's stagnation point flow experiment and Hladovec's net experiment, Lewis demonstrated similarities between milk and blood clotting in flow situations. Petschek's experiment involved the

impingement of a jet of fluid on the back side of a coverslip, with continuous microscopic monitoring (4). Lewis found that both blood and renneted milk deposited in similar fashions, ultimately leading to either a symmetric clot about the stagnation point, or a wedge-shaped clot around imperfections in the glass (5). Hladovec and Ríha's test involved the pumping of fluid through a fine mesh, with continuous monitoring of the upstream pressure (6). Both blood and milk formed a clot in the wake of the mesh, rather than on the upstream, or sieving, side. Additionally, both blood and milk displayed three distinct phases of clotting: an induction phase, with negligible pressure change; a period of gradual pressure increase with visible flocculation; and finally, a sharp pressure rise with the formation of a bulk clot (5).

Encouraged by the results of these tests, Lewis and MacLeod began to look at the possibility of using renneted milk to assess mechanical heart valves. They presented results of studies conducted with Starr-Edwards and Björk-Shiley valves that showed similar-

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ities between thrombus locations in vivo and the sites of localized milk clotting when the valves were run in an artificial heart chamber (3). Subsequently, researchers at the University of Sheffield investigated milk clotting on Björk-Shiley, CarboMedics, and tissue valves (7), with the valves mounted in a straight-walled cylindrical chamber. Again, similarities were found between milk clot location in vitro and thrombogenic sites of the valves in patients.

That milk and blood clot in similar ways in similar fluid dynamic environments should not be so surprising; the action of rennet on  $\kappa$ -casein in milk is similar to that of thrombin on fibrinogen (2,8). Both involve the enzymatic cleavage of a macropptide to form a soluble glycopeptide and insoluble peptide strands. These peptide strands then rapidly polymerize to form a gel matrix. The growth of this coagulum is highly dependent on local fluid dynamics.

The milk test has been proposed to mimic the fluid mechanical causes of clot formation. The heart valves are tested in a model heart chamber, with flow parameters adjusted to give the same Reynolds number and frequency parameter as for blood in the human heart. Since the milk clot grows over time, affecting the local fluid dynamics, the milk test should be able to model the dynamic growth of clot on an artificial heart valve in a way that fluid mechanical tests with clean valves cannot.

In order to assess further the viability of using renneted milk for the detection of potential clotting sites, it was necessary to ascertain whether the milk test would properly predict clotting on all possible valve types. The aim of the present study was to test a wide range of commercial valves in the existing heart chamber for comparison with reported thrombus locations of these valves in vivo. This study includes results for caged-ball valves (Starr-Edwards cloth-covered and silastic ball), tilting-disc valves (Björk-Shiley Standard,

Björk-Shiley Monostrut, Medtronic Hall, Ultracor), and bileaflet valves (St. Jude Medical, CarboMedics, and Edwards-Duromedics).

## Materials and methods

### Experimental set-up

The test rig used in these investigations was similar to that described previously by Christy and Marosek (9); a flow diagram is shown in Figure 1. The rig consisted of a milk preparation section, where the milk was heated and degassed, and a test chamber, where the valves were mounted. Warm water circulating through a parallel plate heat exchanger was used to heat the milk to 37°C for all runs. After heating, and immediately upstream of the test chamber, the milk was split into two separate streams; diluted rennet was added to one stream and saturated aqueous calcium chloride was added to the other. These milk streams were then split again and recombined inside the test chamber to promote mixing of the reagents. Following passage through the test chamber, flow was directed into a drain for disposal.

### Test chamber

The same test chamber as in Lewis' research (3,5) was used in the present studies. This consisted of a rigid, Perspex model of the left side of the human heart (Fig. 2). The mitral section was designed by Lewis and MacLeod based on the hydraulic radii of the left atrium and left ventricle of an average adult. The aortic section was based on a design by Wieting et al. (10), with an ellipsoidal section downstream of the valve for modeling the sinuses of Valsalva. Scaled drawings of the wetted perimeter of the test chamber are shown in Figure 3. Flow was directed into the test chamber via four distinct inlets in the model left atrium, representing blood return from the pulmonary arteries. This

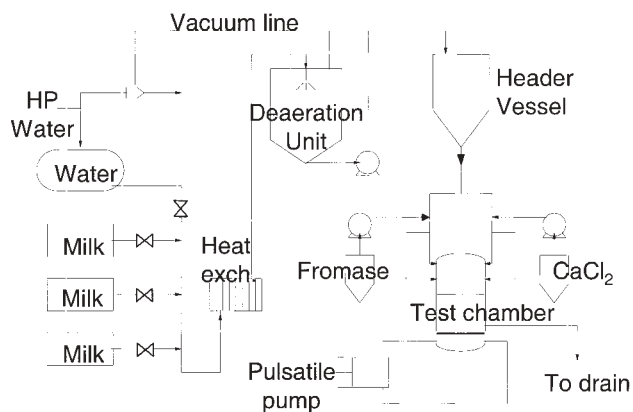


Figure 1: Flow diagram for the milk rig.

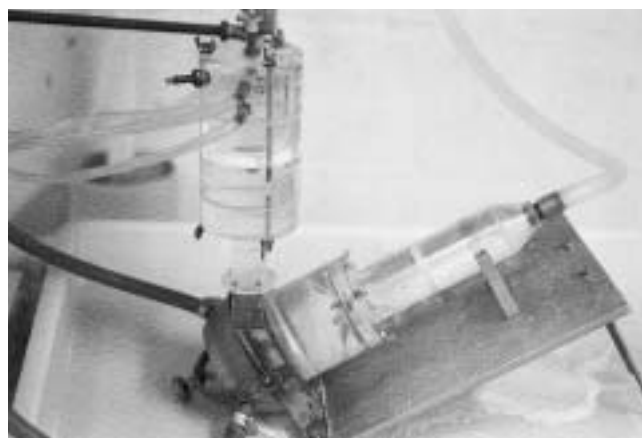


Figure 2: Rigid heart chamber used in the current studies. The aortic valve is mounted in the section on the right.

facilitated mixing of the milk streams in the heart chamber, promoting coagulation. Pulsatile flow through the chamber was accomplished by the use of a diaphragm at the bottom of the left ventricle. Water on the reverse side of the diaphragm was displaced by a piston-driven pulsatile pump, causing the space behind the diaphragm to expand and collapse, moving fluid through the valves. The outlet from the aortic section of the test chamber was positioned above the test chamber to create sufficient back-pressure to close the mitral valve during systole.

All valves were placed in a silicone mold (Silastic RTV 9161), 50 mm diameter, 7 mm thickness. This mold was then mounted in the chamber, sealing the valve in place. For valves with a sewing ring attached, the mold encapsulated the ring, so that all valves had a similar mounting when placed inside the test chamber. It was decided that the inner valve diameter, through which fluid passed, was the most critical for comparison purposes. These were measured with a micrometer, and are listed in Table I. (Note that these are not the same as the tissue annulus diameters reported by valve manufacturers.)

### Milk preparation

Fresh, unpasteurized milk was obtained from the Edinburgh University farm on the day of the run, and pumped through the system at 2 l/min. Streams of rennet and calcium chloride were added to the milk at

Table I: Inner flow diameter of valves tested in the current study.

Heart valve	Diameter (mm)
Caged-ball valves	
Starr-Edwards cloth-covered	19.8
Starr-Edwards silastic ball	19.1
Tilting-disc valves	
Björk-Shiley Standard	24.0
Björk-Shiley Monostrut	22.0
Medtronic Hall	24.0
Ultracor	24.0
Bileaflet valves	
St. Jude Medical	22.4
CarboMedics	20.5
Edwards-Duromedics	24.5

20 ml/min, each representing 1% (by volume) of the total flow. A microbial rennet (Fromase; DSM Food Specialties) was used in all tests. A stock Fromase suspension was diluted to 10% of its original strength with distilled water to obtain the same clotting time used by Lewis (5) of ~45 s at 37°C when mixed with saturated aqueous calcium chloride. Since the residence time of the milk inside the test chamber was only ~20 s, bulk clotting occurred downstream of the test chamber in the drain, whilst only localized clotting was found on the valves.

For the present studies, injection of rennet and calcium chloride continued for 30 min, thus exposing the valves to 60 l of renneted milk. After the 30-min test, flow was switched to water to flush out the test chamber and halt clotting. The test chamber was then dismantled and valves were removed for observation. All valves were photographed using a Nikon F3 SLR camera.

### Cleaning protocol

Careful cleaning of the test rig was necessary after each run in order to prevent any milk clots from carrying over to the next test. With the test chamber disassembled, macroscopic clots were removed from the inside of the chamber using a soft brush. Similar clots were removed from the valves by hand. The test chamber was then reassembled with the valves in place and hot water (~50°C) was run through the system for approximately 30 min to flush out any loose milk clots. When the majority of the clots had been removed, the test rig was split into two sections: the upstream part, which had no contact with rennet; and the downstream part, which had contacted rennet. The upstream section was cleaned with concentrated sodium hydroxide, while the downstream section was

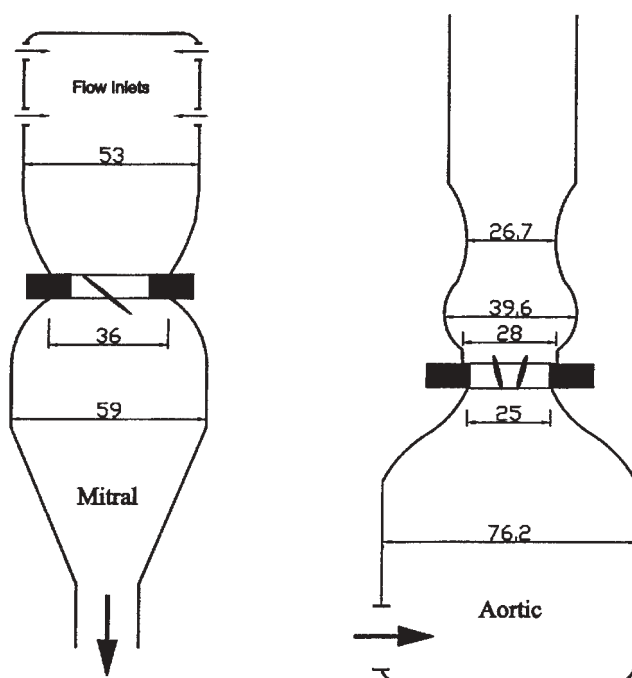


Figure 3: Wetted perimeter of the mitral chamber (left) and aortic chamber (right) showing the valves mounted in place. These drawings are to scale.

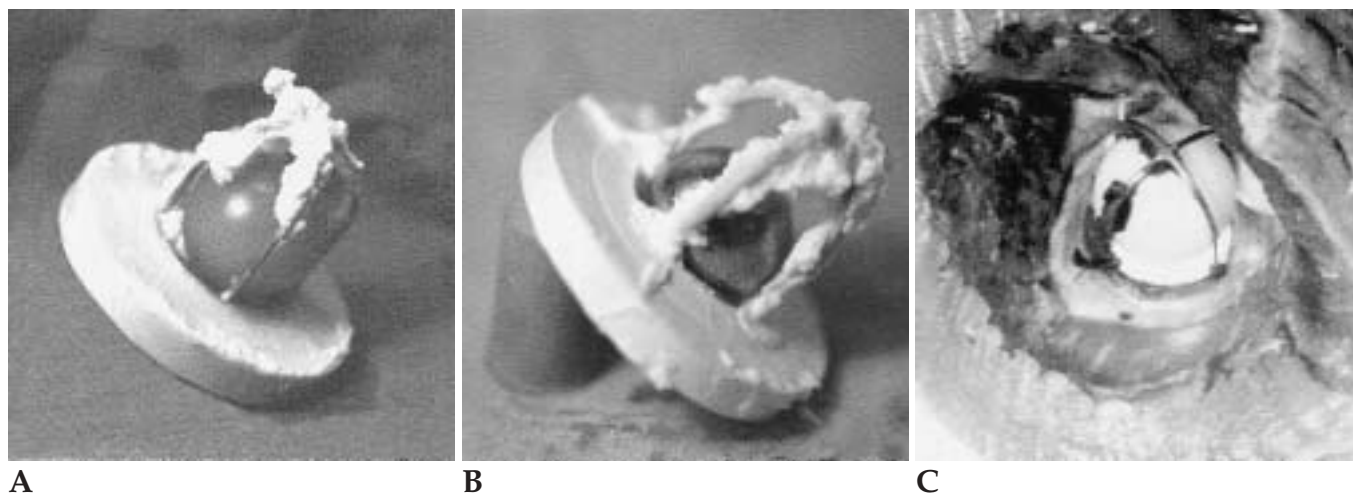


Figure 4: The Starr-Edwards valve. a) Milk clot on the bare strut, silastic ball valve. b) Milk clot on the cloth-covered strut, metal ball valve. c) Thrombus on a Starr-Edwards valve in vivo, showing clot on struts. (Photographs courtesy of Stovin.)

cleaned with the enzymatic agent Tergazyme. Both sections were set up with their respective cleaning agents recirculating through them for at least 3 h. After that time, the system was returned to continuous flow and the Tergazyme and caustic solution were pumped out. Hot water was then flushed through the system for at least 1 h to ensure total removal of the cleaning agents. Appropriateness of this cleaning procedure was confirmed by the similarity in clotting patterns on valves for both their initial and subsequent runs.

## Results

All valves were tested at least three times in both the mitral position and the aortic position of the heart chamber. While clotting occurred in both positions, localized coagulation in the aortic position was considerably more extensive, facilitating comparisons with thrombosis in vivo. The results presented herein were all obtained from valves mounted in the aortic position.

### Caged-ball valves

Two types of caged-ball valve were tested: the Starr-Edwards silastic ball with bare struts, and the Starr-Edwards metal ball with cloth-covered struts; milk-clotting results are shown in Figure 4a and b. For both valves, clot occurred on the struts, with a thickened clot at the apex, often extending downstream of the valve. Frequently, there was also a thinner clot on the lower part of the struts, at the intersection of strut and sewing ring.

Figure 4c shows an explanted Starr-Edwards valve for comparison with the milk-tested valve. With both milk and blood, significant clot can be seen on the struts. Other studies have noted similar thrombus for-

mation, both at the intersection of strut and valve housing and at the downstream apex (11,12).

### Tilting-disc valves

Four different tilting-disc valves were tested: the Björk-Shiley Standard, Björk-Shiley Monostrut, Medtronic Hall, and Ultracor valves. All of these valves have free-floating discs, restrained by and pivoting around struts extending from the orifice ring. For the first three valves, the main clotting occurred downstream, primarily on valve struts. However, the Ultracor valve, which has a large strut extending into the main flow orifice, also had a significant clot upstream. Figure 5a-h shows milk clot on these valves.

### Björk-Shiley valves

The Björk-Shiley Standard and Monostrut valves behaved similarly in the milk test. The thickest clot was on the struts. On the Standard valve, this often extended into the minor orifice region and out to the pivot points. Additionally, a thinner clot frequently formed on the disc itself. The amount of clot present on the disc seemed to depend on whether or not the disc continued to rotate throughout the test. If the disc did rotate, there tended to be clot both upstream and downstream on the center of the disc, but none on the outer edge of the disc, which would have been constantly wiped by the struts. However, if the disc stopped rotating, then there tended to be a covering over the entire disc. There were no cases where the Björk-Shiley Monostrut valve appeared to have stopped rotating; in all instances, the clot on the disc was confined to the disc center.

Several studies have discussed thrombus formation on the Björk-Shiley Standard valve (13-17). In most cases, the valve had clotted completely by the time

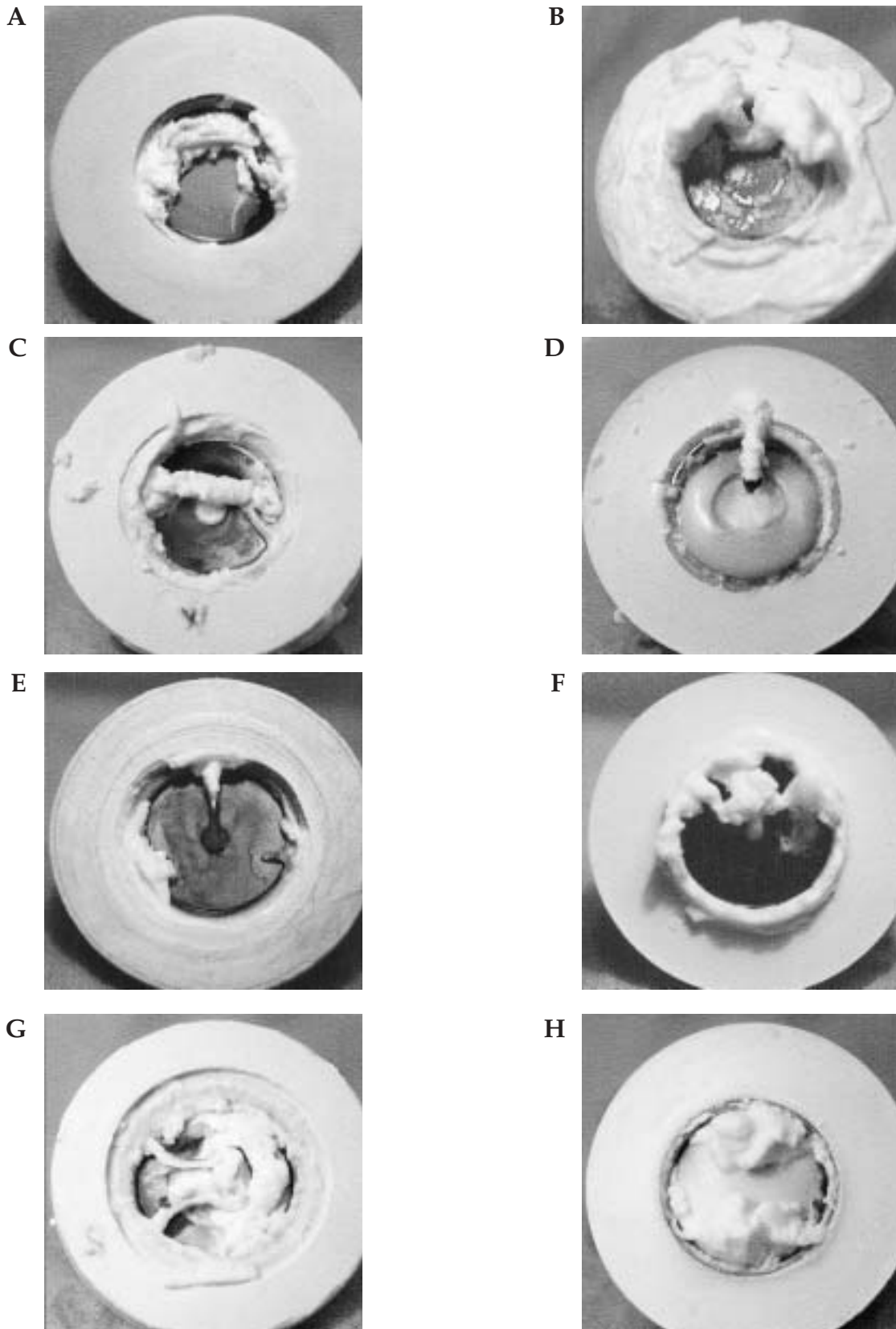


Figure 5: Milk clot on the tilting disc valves. a, b) Upstream and downstream views of the Björk-Shiley Standard valve. c, d) Upstream and downstream views of the Björk-Shiley Monostrut valve. e, f) Upstream and downstream views of the Medtronic Hall valve. g, h) Upstream and downstream views of the Ultracor valve.

thrombus was detected, but some photographs have shown the thrombus at its early stages, providing an indication of clot origin (14,15). Thrombus was typically found on the struts both upstream and downstream, extending to varying degrees into the minor orifice. There is also evidence of thrombus forming at the downstream pivot points (14). Figure 6 shows an explanted valve with thrombus evident on the minor orifice region, for comparison with a valve tested with milk.

#### *Medtronic Hall valve*

The Medtronic Hall valve clotted upstream on the two side projections and at the base of the center strut. There was also a thin clot that formed on the disc itself. Downstream, a significant amount of clot formed on the two struts, with a concentration of clot in the space between them. Clot also formed behind the pivot points, sometimes connecting to the clot formed between the struts in the center.

Burgess et al. (18), Starek et al. (19), and Sharma et al. (20) discussed thrombosis of the Medtronic Hall valve. Figure 7 shows a picture of the upstream side of the thrombosed valve from the Burgess case. Thrombus can be seen extending from the retaining mechanisms at the pivot points in toward the center of the disc. A similar photo can be found in the report by Sharma et al. (20). These thrombi compare with the milk clot found on the retaining mechanisms in the milk test.

#### *Ultracor valve*

The Ultracor valve appeared to be affected by disc

rotation. There was extensive clot found upstream on the region where the struts contacted the disc, and downstream there were thick clots on the disc behind the pivot points. Had the disc been rotating, this clot should not have developed as the pivot points on the disc would have been constantly changing. There is little detailed information available in the literature regarding thrombosis of the Ultracor valve, although there are reported cases of its occurrence (21,22), including a mention of the thrombus causing the disc to stick.

#### **Bileaflet valves**

Three bileaflet valves were tested: St. Jude Medical, CarboMedics, and the Edwards-Duromedics. There was little difference in the clotting between them, despite the slight variations in design. Figure 8a-f shows milk clot on the bileaflet valves.

#### *St. Jude Medical valve*

The St. Jude Medical valve clotted upstream at the hinges on both sides of the leaflets, and downstream behind the hinges. In one case, the downstream milk clot also extended to the downstream outer edge of one leaflet, significantly impairing leaflet motion. There were other cases where the clot behind the hinges decreased the leaflet opening angle before the test was completed. There was also a light coating on the leaflets themselves, although this differed in nature from the localized, more adherent clot on the sides.

Thrombosis around the hinge mechanism of the St. Jude Medical valve is a commonly reported problem

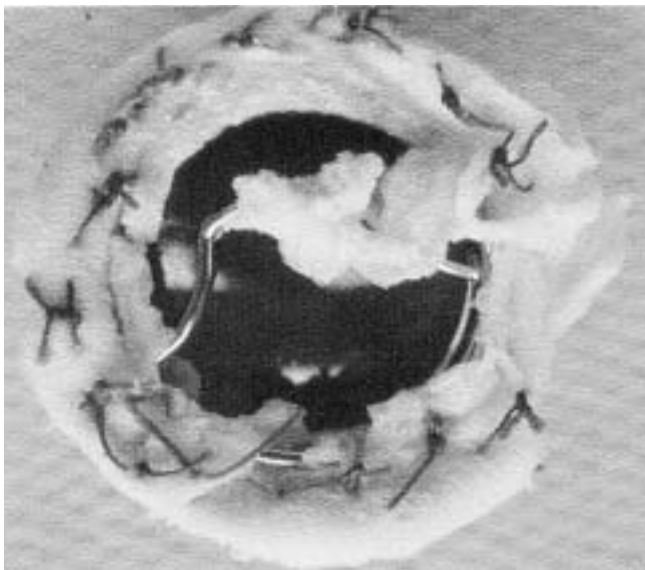


Figure 6: Upstream view of an explanted Björk-Shiley valve, showing thrombus on the struts extending toward the minor orifice. (Courtesy Macgregor.)

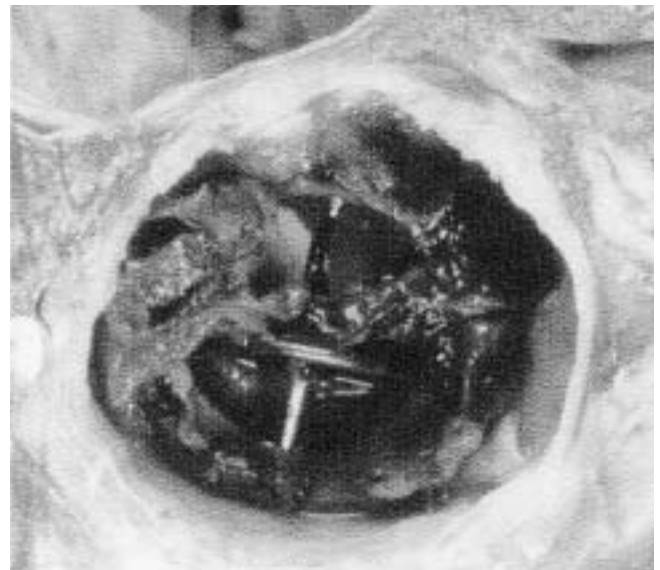


Figure 7: Downstream view of thrombosed Medtronic Hall valve, taken from the level of the aortic root (18). Note the clot extending from the sides toward the center of the valve, for comparison with Figure 5f.

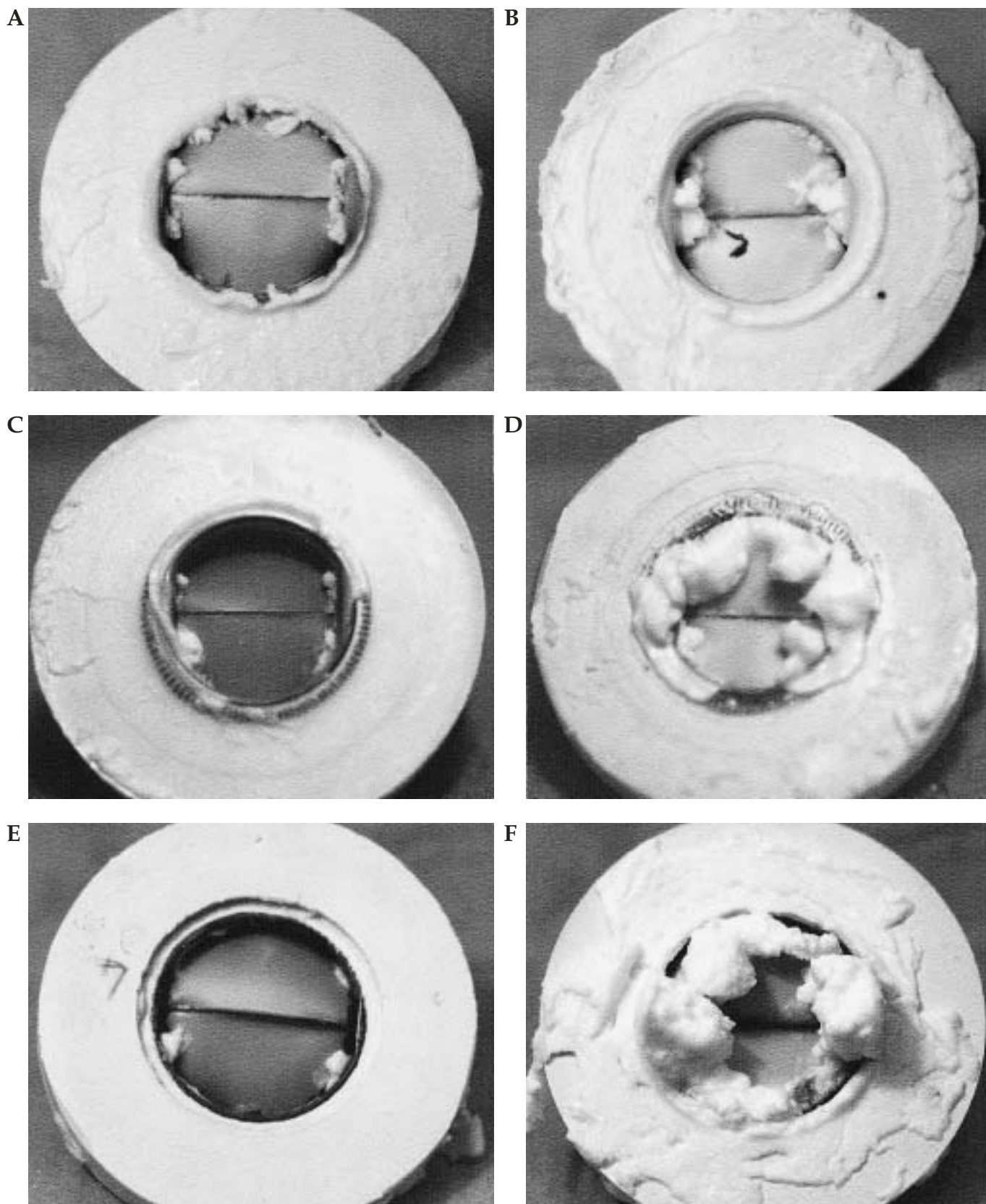


Figure 8: Milk clot on the bileaflet valves. a, b) Upstream and downstream views of the St. Jude Medical valve. c, d) Upstream and downstream views of the CarboMedics valve. e, f) Upstream and downstream views of the Edwards-Duromedics valve.

(23-26). Figure 9 shows an explanted valve with thrombus at the hinges. Similar photographs of thrombus formation can be found elsewhere (23,26,27). It is also common for the thrombosed valve to show decreased leaflet motion, as was found with the milk clot.

#### *CarboMedics and Edwards-Duromedics valves*

The CarboMedics and Edwards-Duromedics valves performed almost identically in the milk test. Both clotted upstream near the hinges and then downstream on the sides behind the hinges. There were also cases with both valves where a large clot formed along the downstream outer edge of one or both leaflets, as shown in Figure 8d and f.

Thrombus occurring around the hinge mechanisms of both the CarboMedics and Edwards-Duromedics valves has been reported in the literature (24,28-30). A similar thrombus was documented by Hirsch and Soldky (28). In some cases, a large thrombus also built up behind the leaflets, interfering with leaflet motion, as happened in the milk test.

## Discussion

The use of milk as a blood analog has often been met with skepticism in the past, due to the different natures of initiation of the clotting reactions. The aim of the present study was to show that, despite those differences, the final product resulting from renneted milk resembles that of blood under similar flow conditions. As this is the case, the nature of activation may not be

critical in modeling the flow-induced thrombosis of heart valves.

Studies have shown that patients with native heart disease (as heart valve recipients generally are) have an altered blood chemistry, with a potentially hypercoagulable state (31,32). Additionally, Goldsmith et al. (32) found that the implantation of a mechanical heart valve increased platelet activation, whereas the implantation of a bioprosthetic valve did not. Koppensteiner et al. found a slight increase in platelet aggregation levels after the implantation of a bioprosthetic valve, but a much larger increase occurred with mechanical prostheses (33). This corresponds with the increased thrombogenic nature of mechanical valves compared to bioprosthetic ones. Since the blood tested in those studies was removed from a vein distant to the heart, the results indicate that for all patients with mechanical heart valves, blood may be in a hypercoagulable state. If that is true, then modeling the mechanisms of aggregation, rather than activation, may be justified.

Still other skeptics have suggested that the milk test merely identifies regions of stasis. However, studies conducted by Christy and MacLeod (34) have indicated that stasis alone is not sufficient for explaining the clot found around the heart valves. In studies with bodies of revolution, these authors noted that deposition did not occur uniformly along the full length of a stagnation zone. To investigate this further, Christy and MacLeod performed modified Lee-White tests and found that agitation of the milk mixture following an induction period resulted in preferential clot deposition at the solid-liquid interface of a test tube wall, while tests without agitation did not show similar deposition. Thus, agitation was clearly a factor in the source of clot development. Further investigations by Christy and Marosek showed that milk clot deposited on the wall of a test chamber in pulsatile flow differed considerably from that formed in steady flow: the pulsatile-flow wall clot was more adherent and denser than the steady-flow wall clot (9). The difference in nature between the two types of wall clot was attributed to differences in shear forces between the flow regimes. In pulsatile flow, the wall shear forces were significantly greater than those present in steady flow. It is therefore probable that shear forces also affect milk clotting of heart valves.

Extensive investigations have been conducted to characterize the flow fields around prosthetic heart valves, but it has not been possible to use this information to rank valves according to their thrombogenic potential (35,36). One reason for this is that there may actually be little difference between the valves commercially available today. A review of the literature quickly exposes the discrepancies in reports of valve

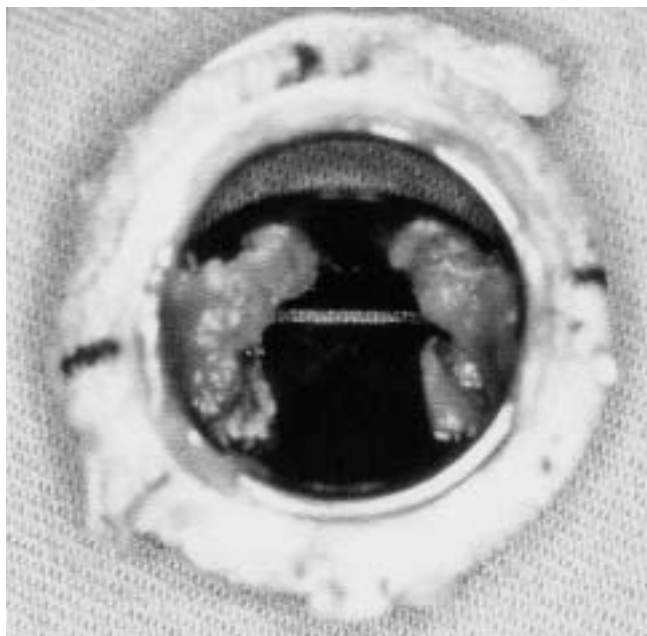


Figure 9: Thrombi on the bileaflet valves, for comparison with milk clot formation. Clot around the hinges of the St. Jude Medical valve. (Photograph courtesy of T. Ohata.)

thrombogenicity. Mikaeloff et al. found that the St. Jude Medical valve had a significantly lower thromboembolic rate than the Björk-Shiley and the Starr-Edwards valves (37). In Fiore et al.'s study, the St. Jude Medical and Medtronic Hall valves performed identically (38), yet a group in the Netherlands found the Medtronic Hall valve to be significantly worse than both the Björk-Shiley valve and the Duromedics bileaflet (39). As there is no clear indication that one of these valves performs better than another *in vivo*, it is difficult to correlate one specific flow feature with a valve's thrombogenic potential.

### Comparison with previous studies

All valves tested in the aortic position produced distinctive, reproducible clotting patterns. The locations of clot formation compared favorably with those presented previously (3,7). However, the extent of clotting downstream of the CarboMedics valve in this test tended to be greater than that found in Keggen's studies, and more similar to the thrombosed valve presented by Keggen et al. (7). (This valve is included as Fig. 9b of this paper, courtesy of Patricia Lawford.) The differences in clot extent may be due to the different geometries of the test chambers. The aortic chamber in this study included a recessed ellipsoidal section for modeling the sinuses of Valsalva, while Keggen et al.'s study involved a simple cylindrical chamber. Morsi et al. showed that the downstream flow field for a chamber with an ellipsoidal sinus differs from that of a cylindrical chamber (40), so this difference may have affected the extent of clot formation.

### Comparison between milk and blood clotting

For the ball valves, primary milk clotting occurred on the struts, was thickest at the apex, and frequently trailed downstream. The struts have also been associated with thrombosis *in vivo*, sometimes interfering with poppet motion (41). Additionally, caged-ball valves have been associated with a relatively high rate of embolism (42,43), indicating that additional clot forming on the struts may be wiped free whenever the heart contracts. Since the current heart chamber in the milk test has rigid walls, this same phenomenon cannot be observed.

The struts on the tilting-disc valves - particularly any retaining parts downstream of the disc - were also associated with clotting. Additionally, milk clot developed downstream of the actual disc pivot points, where stagnation would have occurred. For the Björk-Shiley Standard and Medtronic Hall valves, it was possible to compare milk clot locations with thromboses found *in vivo*. In both cases, there were striking similarities between the two. For the Ultracor valve, however, there was little information on clotting of the

valve *in vivo*. It is therefore unknown if the extensive upstream clotting of the valve would actually occur in the human heart. The design of the current heart chamber, with its rigid walls and bottom diaphragm, establishes a vorticity in the left ventricular chamber that would not be present in the native heart. For most valves, this would have little effect on the ultimate flow pattern through the aortic position. However, since the Ultracor valve has a significant strut that extends into the major flow orifice upstream of the valve, it is hypothesized that the swirling motion of the fluid prior to entering the valve could cause an unrepresentative amount of clot to form on the upstream strut. More knowledge of the thrombogenic nature of the Ultracor valve is needed to allow comparison of the milk clot on this valve with what happens *in vivo*.

There were few obvious differences between the clotting of milk on the bileaflet valves, with all valves clotting around the hinge mechanism, primarily on the downstream edge. Leaflet motion was sometimes impaired by the development of a bulky clot behind the hinge housing, between the leaflets. Similar problems have been reported for various bileaflet valves *in vivo* (24,44).

The fact that all valves had localized regions of milk clotting indicates that no valve design is yet ideal. While the first-generation Starr-Edwards and Björk-Shiley valves clotted primarily around any struts protruding into the flow orifice, the bileaflet valves clotted around the hinge mechanism. This has also been noted in a review of valve hemodynamics and thrombosis by Giddens et al. (36). The design of the Medtronic Hall valve, while providing a larger effective opening area and less flow separation, did not eliminate incidences of thrombosis. Nor did the introduction of bileaflet valves which, while also providing larger flow areas, has increased regurgitation and flow separation around the hinges. At present, all available mechanical valves are still subject to thrombosis, and this was reflected in the milk clot found around the valves *in vitro*.

### Limitations of the existing system

There are areas of the existing system that need to be addressed. As mentioned previously, a non-physiological swirling motion developed in the left ventricular chamber which may have affected downstream clotting of valves in the mitral position, and upstream clotting of valves in the aortic position. Little localized clotting occurred on valves in the mitral position of the test chamber, although this is the position known to be more thrombogenic in clinical practice. This was due in part to the fact that the renneted milk was at an earlier stage in the clotting process when it encountered the mitral valve, but it may also have resulted from the

inherent rigid nature of the test chamber. A compliant model is being developed that should more accurately model true physiological conditions. Another aspect of the current milk test that needs to be revised is the sinusoidal nature of the pulsatile pump. It is recognized that a sharper spike may be needed to fully open some valves. Assessment with a true cardiovascular waveform will be completed in the future.

Another limitation of the current system was the inability for visualization of clot development during the test. At present, it is only possible to observe clot that has formed at the end of the run, after the test has been halted and the test chamber disassembled. It would be most beneficial to actually monitor the time course of clotting throughout a run. Previous studies at the present authors' institute have shown promise with the use of ultrasound for continuous monitoring (9); however, these investigations were conducted using a cylindrical chamber with constant wall thickness. Extrapolation of that technique to the complex geometry of the existing heart chamber has not been possible. However, there is still the possibility of stopping a test at different time intervals to obtain information about the origin of clotting, an advantage not practical in animal trials.

Currently, it is possible to compare the *locations* of milk clot to those of thrombus formation, but not the *extent* of clotting. As discussed by Keggen et al. (7), one reason for this is that the milk test is conducted over a fixed time period (30 min), whereas a thrombosed valve may be detected at any point in the clotting process. In fact, the thrombus is often only detected when it causes catastrophic failure. However, the extent of clot that is sufficient to cause failure varies significantly for different types of valves. Tilting-disc valves have long been associated with sudden and acute failure due to thrombosis of the minor orifice (12). Even smaller thrombi can interfere with bileaflet valve function (23). On the other hand, a caged-ball valve may continue to function long after thrombus has developed (45). Thus, a miniscule thrombus on a bileaflet valve that has caused a leaflet to stick may be detected early in the clotting process, whereas a thrombus on the struts of a caged-ball valve may never be detected in a patient's lifetime (45).

*In conclusion*, the aim of these studies was not to rank the valves according to thrombogenic potential. Indeed, there would be no grounds for making that comparison since such contradictory evidence exists in the literature as to which valves truly are more prone to clotting than others (37-39). Additionally, since the extent of clotting that may be catastrophic varies for valve type, assessing a valve based purely on the quantity of clot formed is not an appropriate method of

evaluation. However, since all thrombi have the potential to embolize or suddenly cause acute heart failure, it is important to be able to identify the locations prone to clotting. The current results are intended to show that a renneted milk test has the ability to identify such thrombogenic locations; all valves for which clinical data were available clotted with renneted milk in the same locations where thrombus has been found in vivo. The ability of the milk test to detect accurately any thrombogenic sites in existing heart valves implies its ability to predict thrombogenic sites in valve prototypes. Thus, the milk test could serve as an inexpensive test on the road to heart valve development.

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### References

1. Bodnar E. The Medtronic Parallel (TM) valve and the lessons learned. *J Heart Valve Dis* 1996;5:572-573
2. Jollès P. Structural aspects of the milk clotting process. Comparative features with the blood clotting process. *Mol Cell Biochem* 1975;7:73-85
3. Lewis JMO, MacLeod N. A blood analogue for the experimental study of flow-related thrombosis at prosthetic heart valves. *Cardiovasc Res* 1983;17:466-475
4. Petschek H, Adamis D, Kantrowitz AR. Stagnation flow thrombus formation. *Trans Am Soc Artif Intern Organs* 1968;14:256-259
5. Lewis JMO. A blood analogue for thrombogenicity assessment. PhD thesis, University of Edinburgh, 1981
6. Hladovec H, Ríha P. The model of thrombosis and thrombolysis in vitro. *Thromb Res* 1975;7:743-752
7. Keggen LA, Black MM, Lawford PV. The use of enzyme-activated milk for in vitro simulation of prosthetic valve thrombosis. *J Heart Valve Dis* 1996;5:74-83
8. Payens TAJ, Wiersma AK, Brinkhuis J. On enzymatic clotting processes I. Kinetics of enzyme-triggered coagulation reactions. *Biophys Chem* 1977;6:253-261
9. Christy JRE, Marosek KW. Ultrasonic determination of clot deposition rates in a milk-based, in-vitro procedure for thrombogenicity assessment. *J Heart Valve Dis* 2000;9:379-388

10. Wieting DW, Hall CW, Liotta D, Bakey MED. Dynamic flow behavior of artificial heart valves. In Brewer LA (ed.), *Prosthetic heart valves*. C C Thomas, Springfield, IL, 1969:34-51
11. Miller DC, Oyer PE, Mitchell RS, et al. Performance characteristics of the Starr-Edwards model 1260 aortic valve prosthesis beyond ten years. *J Thorac Cardiovasc Surg* 1984;88:193-207
12. Metzdorff MT, Grunkemeier GL, Pinson CW, Starr A. Thrombosis of mechanical cardiac valves: A qualitative comparison of the silastic ball valve and the tilting disc valve. *J Am Coll Cardiol* 1984;4:50-53
13. Harjula A, Mattila S, Maamies T, et al. Long-term follow-up of Björk-Shiley mitral valve replacement: 10 years' experience. *Scand J Thorac Cardiovasc Surg* 1986;20:79-84
14. Björk VO, Henze A. Management of thromboembolism after aortic valve replacement with the Björk-Shiley tilting disc valve. *Scand J Thorac Cardiovasc Surg* 1975;9:183-191
15. Moreno-Cabral RJ, McNamara JJ, Mamiya RT, Brainard SC, Chung GKT. Acute thrombotic obstruction with Björk-Shiley valves. *J Thorac Cardiovasc Surg* 1978;75:321-330
16. Copans H, Lakier JB, Kinsley RH, Colsen PR, Fritz VU, Barlow JB. Thrombosed Björk-Shiley mitral prostheses. *Circulation* 1980;61:169-174
17. Ben-Zvi J, Hildner FJ, Chandraratna PA, Samet P. Thrombosis on Björk-Shiley aortic valve prosthesis. *Am J Cardiol* 1974;34:538-544
18. Burgess M, Millane T, Deiraniya A. Acute thrombosis in an aortic prosthesis: All mechanical valves are not the same. *Hosp Med* 2001;62:788-789
19. Starek PJK, Beaudet RL, Hall K-V. The Medtronic-Hall valve: Development and clinical experience. *Cardiac Surg* 1987;1:223-236
20. Sharma N, Grover A, Radotra BD. Prosthetic cardiac valve replacement: Management problems. *Asian Cardiovasc Thorac Ann* 1998;6:179-182
21. Li H-H, Jeffrey RR, Davidson KG, Seifert D, Körfer R, Grunkemeier GL. The Ultracor tilting disc heart valve prosthesis: A seven-year study. *J Heart Valve Dis* 1998;7:647-654
22. Tasdelen A, Ikizler C, Aslamaci S, Yavari A, Ekici E, Arslan G. Three-year experience with the Ultracor valve prosthesis. *Asian Cardiovasc Thorac Ann* 1998;6:265-269
23. Nuñez L, Iglesias A, Sotillo J. Entrapment of leaflet of St. Jude Medical cardiac valve prosthesis by miniscule thrombus: Report of two cases. *Ann Thorac Surg* 1980;29:567-569
24. Kayali MT, Fetieh MW, Abdulsalam MA, Memon F, Moinuddin M, Raffa H. Thrombotic obstruction of bileaflet mechanical prosthetic heart valves: Early diagnosis and management. *J Cardiovasc Surg* 1998;39:331-335
25. Al-Halees Z, Kumar N. Thrombotic obstruction of bileaflet valves: Surgical management and fiberoptic thrombectomy. *Ann Thorac Surg* 1994;58:168-169
26. Bowen TE, Tri TB, Wortham DC. Thrombosis of a St. Jude Medical tricuspid prosthesis. *J Thorac Cardiovasc Surg* 1981;82:257-262
27. Prabhu S, Friday KJ, Reynolds D, Elkins R, Lazzara R. Thrombosis of aortic St Jude valve. *Ann Thorac Surg* 1986;41:332-333
28. Hirsch R, Soldky A. Recurrent thrombosis of bileaflet prosthetic valves. *Circulation* 1996;93:2088
29. Montorsi P, Cavoretto D, Ballerini G. Thrombosis of mechanical heart valve prostheses: Revisiting the role of fluoroscopy. *Br J Radiol* 2000;73:76-79
30. Deuvaert FE, Dumont N, Primo GC. Fluoroscopic differentiation between leaflet escape (LE) and valve thrombosis (VT) of the Edwards-Duromedics mitral valve. *Acta Cardiol* 1989;44:221-228
31. Koppensteiner R, Moritz A, Moidl R, et al. Blood rheology in patients with native heart valve disease and after valve replacement. *Am J Cardiol* 1998;81:250-252
32. Goldsmith IRA, Blann AD, Patel RL, Lip GYH. Effect of aortic valve replacement on plasma soluble p-selectin, von Willebrand factor, and fibrinogen. *Am J Cardiol* 2001;87:107-110
33. Koppensteiner R, Moritz A, Schlick W, et al. Blood rheology after cardiac valve replacement with mechanical prostheses or bioprostheses. *Am J Cardiol* 1991;67:79-83
34. Christy JRE, MacLeod N. The role of stasis in the clotting of blood and milk flows around solid objects. *Cardiovasc Res* 1989;23:949-959
35. Walker PG, Yoganathan AP. In vitro pulsatile flow hemodynamics of five mechanical aortic heart valve prostheses. *Eur J Cardiothorac Surg* 1992;6(suppl.1):S113-S123
36. Giddens DP, Yoganathan AP, Schoen FJ. Prosthetic cardiac valves. *Cardiovasc Pathol* 1993;2:167S-177S
37. Mikaeloff P, Jegaden O, Ferrini M, Coll-Mazzei J, Bonnefoy JY, Rumolo A. Prospective randomized study of St. Jude Medical versus Björk-Shiley or Starr-Edwards 6120 valve prostheses in the mitral position. *J Cardiovasc Surg* 1989;30:966-975
38. Fiore AC, Barner HB, Swartz MT, et al. Mitral valve replacement: Randomized trial of St. Jude and Medtronic Hall prostheses. *Ann Thorac Surg* 1998;66:707-713
39. Kuntze CEE, Blackstone EH, Ebels T. Thromboembolism and mechanical heart valves: A randomized study revisited. *Ann Thorac Surg* 1998;66:101-107
40. Morsi YS, Sakhaeimanesh A, Clayton BR. Hydrodynamic evaluation of three artificial aortic

- valve chambers. *Artif Organs* 2000;24:57-63
41. Dale J. Arterial thromboembolic complications in patients with Starr-Edwards aortic ball prostheses. *Am Heart J* 1976;91:653-659
  42. Levine FH, Copeland JG, Morrow AG. Prosthetic replacement of the mitral valve: Continuing assessments of the 100 patients operated upon during 1961-1965. *Circulation* 1973;47:518-526
  43. Akbarian M, Austen WG, Yurchak PM, Scannel JG. Thromboembolic complications of prosthetic cardiac valves. *Circulation* 1968;37:826-831
  44. Shapira Y, Sagie A, Jortner R, Adler Y, Hirsch R. Thrombosis of bileaflet tricuspid valve prosthesis: Clinical spectrum and the role of nonsurgical treatment. *Am Heart J* 1999;137:721-725
  45. Roberts WC, Hammer WJ. Cardiac pathology after valve replacement with a tilting disc prosthesis Björk-Shiley type. *Am J Cardiol* 1976;37:1024-1033