Aortic Root Characteristics of Human Pulmonary Autografts

Gerald S. Carr-White, MRCP; A. Afoke, PhD; E.J. Birks, MRCP; S. Hughes, MBBS; A. O’Halloran; Sally Glennen, RGN; Sue Edwards, BSc; M. Eastwood, PhD; Magdi H. Yacoub, FRS

Background—After pulmonary autograft replacement of the aortic valve and root, the pulmonary artery (PA) wall is subjected to higher pressures. Concern exists that this may lead to structural and functional changes in the implanted autograft and subsequent aortic root dilatation and neoaortic regurgitation. We therefore assessed root dimensions and neoaortic regurgitation, morphological structure, and mechanical behavior in patients who underwent the Ross operation.

Methods and Results—Seventy-four patients who were randomized to undergo aortic valve replacement with an aortic homograft or a pulmonary autograft were followed up echocardiographically for up to 4 years and had their aortic root dimensions measured at the level of the annulus, sinuses, and sinotubular junction. In a separate series of 18 patients who underwent pulmonary autograft surgery and 8 normal organ donors, samples from the PA and aorta were analyzed for medial wall thickness, distribution of the staining of collagen and elastin, and elastin fragmentation. Finally, stress-strain curves were obtained from samples of the PA and aorta from 9 patients who underwent pulmonary autograft surgery and from 1 patient in whom a 4-month-old autograft was explanted. No patient in either group had aortic dilatation at any level of 20% or more than mild aortic regurgitation at up to 4 years of follow-up. The aortic media was thicker in both autografts and normal donors (P<0.01), and there was a trend for the PA media to be thicker in the autograft group. Elastic fiber in all aortas showed little or no variation, whereas in the PA, there was considerable variation in fragmentation. Patients with higher preoperative PA pressures tended to have lower fragmentation scores (χ² P<0.01). The lower stiffness modulus, higher stiffness modulus, and maximum tensile strength of the aorta was 34% to 38% higher than that of the PA (P<0.01); however, the 4-month-old autograft appeared to show adaptation in mechanical behavior.

Conclusions—In our series of patients, there was no significant progressive dilatation of the aortic root. We demonstrated differences in the anatomic structure and mechanical behavior of the PA in vitro and highlighted histological and mechanical modes of adaptation. (Circulation. 2000;102[suppl III]:III-15-III-21.)

Key Words: aorta ■ valves ■ arteries ■ grafting

The ideal valve substitute for aortic valve replacement remains unclear. The use of biological valves offers several theoretical advantages, including preservation of the normal aortic valve mechanism, preserved distensibility of the aortic root, and superior hemodynamics.1,2 Although free-standing homograft root replacement for replacement of the aortic valve has been shown to be safe and effective in the short and long term,3,4 concerns still remain regarding late degeneration and calcification. The use of a pulmonary autograft has several additional theoretical advantages over homografts; in particular is the ability to grow and the improved hemodynamics and durability.5–7 There are, however, potential disadvantages. In adults, the pulmonary artery (PA) and the aorta are known to differ in structure,6,9 and after pulmonary autograft replacement of the aortic valve and root, the PA wall is subjected to higher pressures. Concern exists that this may lead to structural and functional changes in the implanted autograft and that such changes could lead to progressive aortic root dilatation and neoaortic regurgitation.10,11 Such a concern is backed up by recent reports in the literature.12 The aim of the present study was therefore to examine aortic root characteristics in pulmonary autografts through the use of 3 methods. First, we compared aortic root dimensions and neoaortic regurgitation in similar groups of patients from a subset of a randomized trial that compared pulmonary autografts and aortic homografts. Second, we compared collagen and elastic fiber distribution in the aorta and PA in a series of autograft recipients and a series of normal organ donors. Finally, we investigated the mechanical properties of samples of aortic and PA wall in patients who
underwent pulmonary autograft surgery. The aim of the use of 3 separate methods was to determine the pattern and potential determinants of autograft dilatation.

**Methods**

**Patient Selection**

From May 1994 to July 1998, 74 patients were prospectively randomized to undergo aortic valve replacement with an aortic homograft or a pulmonary autograft as part of a larger ongoing randomized trial. Local ethics committee approval was obtained the study was started, and full informed consent was obtained from each patient. The study included all grades of ventricular function, prior aortic valve replacement, bacterial endocarditis, and emergency operations. Only patients with sequential follow-up with high-quality echocardiograms of the aortic root were included in the study. However, the patient demographics, reason for surgery, and preoperative ventricular function were similar between the 2 groups and are illustrated in Table 1.

**Surgical Technique**

All operations were performed by the same surgeon (M.Y.). The surgical technique has been described elsewhere. Only the operative details relevant to possible autograft dilatation are described here. The aortic valve was exposed through a curved aortotomy that began anteriorly and extended into the middle of the noncoronary cusp or anteriorly and extended into the middle of the noncoronary cusp or facing sinus of the autograft (which is thin and not supported by pericardium) was placed in the left coronary sinus position of the new aortic root. Cross-clamp and bypass times were significantly longer in the autograft group (128 ± 94 vs 15 ± 189 minutes, respectively) than in the homograft group (94 ± 12 and 129 ± 21 minutes, respectively; P < 0.001).

**Follow-Up**

Transthoracic echocardiograms were performed before discharge, at 6 months, and at yearly intervals thereafter with a Hewlett-Packard Sonos 500 or 2000 machine. Aortic valve insufficiency was graded according to the method described by Perry et al. Regurgitation not sufficiently severe to be measured according to these criteria was considered trivial. Fractional shortening (FS) was calculated as $FS = (EDD - ESD)/EDD \times 100,$ where EDD is the end-diastolic dimension and ESD is the end-systolic dimension. Aortic root dimensions were measured at 3 levels: the level of the annulus, the sinuses of Valsalva at the largest anteroposterior diameter, and the sinotubular junction. Measurements were recorded only where 2 independent observers agreed the image quality was sufficient for accurate measurements. Dimensions were measured from parasternal long-axis views of the aortic root with use of the inner wall distance during systole. At each level, the clearest view from 10 consecutive cardiac cycles was taken.

**TABLE 1. Patient Characteristics**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Echo Autograft</th>
<th>Echo Homograft</th>
<th>Morph Autograft</th>
<th>Morph Donor</th>
<th>Mech Autograft</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, n</td>
<td>49</td>
<td>37</td>
<td>18</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>Sex, n</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>42</td>
<td>32</td>
<td>14</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Female</td>
<td>7</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Age at surgery, y</td>
<td>18–64</td>
<td>18–65</td>
<td>6–60</td>
<td>29–65</td>
<td>10–59</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>39 ± 8</td>
<td>40 ± 9</td>
<td>36 ± 14</td>
<td>52 ± 11</td>
<td>34 ± 15</td>
</tr>
<tr>
<td>Previous AVR, n</td>
<td>19</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indication for surgery, n</td>
<td>17</td>
<td>15</td>
<td>7</td>
<td>...</td>
<td>4</td>
</tr>
<tr>
<td>AS</td>
<td>19</td>
<td>13</td>
<td>9</td>
<td>...</td>
<td>4</td>
</tr>
<tr>
<td>AR</td>
<td>13</td>
<td>9</td>
<td>2</td>
<td>...</td>
<td>1</td>
</tr>
<tr>
<td>Bacterial endocarditis</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>...</td>
<td>0</td>
</tr>
<tr>
<td>Postoperative function</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDD, mean ± SD cm</td>
<td>3.4 ± 0.9</td>
<td>3.8 ± 0.8</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>ESD, mean ± SD cm</td>
<td>5.1 ± 1.0</td>
<td>5.4 ± 0.8</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>FS, mean ± SD %</td>
<td>32 ± 7.3</td>
<td>30 ± 8.2</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

*Echo indicates echocardiographic series; Morph, morphological series; Mech, mechanical series; AVR, aortic valve replacement; AR, aortic regurgitation; and AS, aortic stenosis.*
Reproducibility
Echocardiographic variables were assessed by a second blinded observer who reanalyzed images from a random sample of 10% of the beats. The variables assessed were aortic root dimensions at the 3 levels. In a separate group of 10 patients who underwent transesophageal echocardiography (TEE) for clinical indications, transthoracic measurements were compared with simultaneous transesophageal measurements. The root-mean-square difference between duplicate measurements and the values for coefficient of variation derived from the ratio of the root-mean-square difference and absolute value were determined.

Morphological Methods
Samples were taken from the anterior wall of the PA and aorta at the time of surgery. The patient demographics and characteristics of the 2 series (18 patients who underwent pulmonary autograft surgery and 8 normal organ donors) are shown in Table 1. The samples were immediately fixed in 10% Formol saline for 2 to 3 days and then processed with paraffin wax. Sections of 4 μm were cut and dried overnight at 37°C. Sections were stained for elastic tissue with elastic van Geisen. Immunocytochemistry was performed with the primary antibodies collagen 1 and collagen III (monosan) and distribution of staining (area) for collagen types 1 and 3, and elastin was measured with image analysis. Fragmentation of elastic fibers in the pulmonary artery was graded semiquantitatively from 0 (no fragmentation) to 4 (severe).

Mechanical Methods
Specimens were obtained at the time of pulmonary autograft surgery from 9 patients whose demographics are detailed in Table 1 and from a 14-year-old patient in whom a 4-month-old autograft was explanted. Before testing, all soft tissue was removed from the outer surface of the specimens. This was done carefully so as not to damage the surface of the tissue. When the specimens were obtained intact as a ring, the circumference was measured before it was divided into test samples. Samples ~25 mm long were further divided into parallel strips with an especially designed cutter block. The width and thickness of these parallel strips were measured with a Nikon profile projector with 50× magnification. The specimen was mounted in clamps designed to test biological tissues and tested in tension at an extension rate of 10 mm/min. A gauge length of 6 mm was used throughout the experiments. Force was measured with a 50 N load cell, and displacement was monitored with a linear voltage differential transducer. The outputs of both were recorded as force V extension with an x-y plotter. Circumferential stress produced by 200 mm Hg was calculated with the formula δh=(pressure*diameter)/(2*wall thickness).

Statistical Analysis
Statistical analysis was performed with a commercially available software package (SPSS Inc). A comparison of demographic and preoperative data between groups was performed with the use of an unpaired t test. A comparison of data over time was performed with 1-way ANOVA. A comparison of categorical variables was performed with χ² test. A value of P<0.05 was accepted as statistically significant.

Results
Echocardiographic Results
Immediate postoperative dimensions, obtained before discharge, are detailed in Table 2 and Figure 1. Subsequent dimensions for up to 4 years of follow-up are detailed in Table 2. Figure 2 shows aortic root dilatation at the 3 sites for the 2 groups expressed as percent increase from the immediate postoperative dimension. The mean increase in diameters was <10%, with no patient in either group having dilatation at any level of >20%. Those who underwent pulmonary autograft surgery showed a trend of more marked dilatation at the level of the sinotubular junction, although this did not reach statistical significance. Those who received aortic homograft root replacement showed a tendency to have reduced diameters at the level of the sinuses and sinotubular junction at years 2 and 3, although again, this was not statistically significant. Mean grades of aortic regurgitation are shown in Table 2 and Figure 3. No patient in either group had more than mild aortic regurgitation at up to 4 years of follow-up.

![Figure 1](http://circ.ahajournals.org/)

**Figure 1.** Aortic root dimensions obtained within 7 days of surgery, before discharge. Results are expressed as mean±SEM. S-T JUNCT indicates sinotubular junction.
Morphological Results
Table 3 details aortic and PA media thickness plus fractional area of staining for collagen and elastin in the patient and donor groups. The aortic media was thicker in both groups (P<0.01). There was a trend for the PA media to be thicker in the autograft group than in the donor group, but this did not reach statistical significance. In the autograft series, collagens I and III showed more order in the aorta than in the PA but were distributed similarly in both. Elastic fiber in all aortas showed little or no variation, whereas in the PA, there was considerable variation in fragmentation. Figure 4 shows typical examples of this variation. The fragmentation did not correlate with patient age, sex, primary diagnosis, symptom duration, preoperative degree of aortic regurgitation, or previous valve surgery. However, patients with higher preoperative PA pressures tended to have lower fragmentation scores (χ² P<0.01 for normal versus raised PA pressures, Figure 5). In the small number of patients studied, there was no relationship between elastic fiber fragmentation and subsequent aortic root dilatation or development of neoaortic regurgitation.

Mechanical Results
Figure 6 shows a typical stress-strain curve for the aorta and the PA. Both exhibit 2 distinct linear portions; the portion with a low stiffness modulus is linked by a toe region to the portion with a much higher stiffness modulus. The values for the lower stiffness modulus, the higher stiffness modulus, and the maximum tensile strength are shown in Table 4, demonstrating that for all 3 indices, the aorta is 34% to 38% stronger.
than the PA. It is interesting to note that the ratio of the higher modulus to the lower modulus for both the aorta and the PA is similar, namely 27. The values for maximum distensibility are also shown, demonstrating that the PA is more stretchable than the aorta by 10%.

Figure 7 shows the stress-strain curve from a 4-month-old explanted autograft superimposed on typical PA and aortic curves. This demonstrates an increase in stiffness compared with the other PA values, albeit with only a patient number of 1. Also superimposed is the circumferential stress that would be generated by a systolic arterial pressure of 200 mm Hg, demonstrating a level that encroaches on the region of higher stiffness for pulmonary, but not aortic, tissue.

### Reproducibility

The coefficient of variability of the measurements for remeasured traces and TEE comparisons are detailed in Table 5. This demonstrates a higher coefficient of variability for measurements at the level of the sinotubular junction than the other 2 levels.

### Discussion

The results of the present study demonstrate that in patients who receive pulmonary autografts as root replacements, neoaoic regurgitation was not more than mild at 4-year follow-up. This is in keeping with other published results; however, our series also showed no significant progressive dilatation of the aortic root, which is in contrast to previously reported value during a similar time period. Only 10 of the autograft patients had congenital bicuspid aortic valve disease and extended follow-up of 3 years. It is possible that longer follow-up in a larger patient series may yet demonstrate aortic root dilatation. Alternatively, variations in surgical technique, particularly involving the proximal and distal suture line as well as orientation of the autograft, and in patient characteristics may be responsible. Interestingly, the trend in dimensional change differed between autografts and homografts. The homografts tended to show reduced diameters at the level of the sinuses and sinotubular junction at years 2 and 3. This would be in keeping with increased stiffness and subclinical calcification of the homograft root. The present study also demonstrates that the reference range for “normal” pulmonary autograft root diameters lie outside those seen for native aortic roots. The fact that in our series of autograft patients the sinotubular junction diameter was slightly greater than the annulus diameter is in contrast to previous reports and may

### Table 3. Medial Wall Thickness and Fiber Distribution in Normal Donors and Patients Who Underwent Pulmonary Autograft Surgery

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th></th>
<th></th>
<th>Donors</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PA</td>
<td>Aorta</td>
<td>PA</td>
<td>Aorta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medial wall thickness, mm</td>
<td>1.21±0.14</td>
<td>1.55±0.19</td>
<td>0.93±0.13</td>
<td>1.52±0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distribution of collagen type 1, % area</td>
<td>0.62±0.09</td>
<td>0.65±0.08</td>
<td>0.62±0.09</td>
<td>0.65±0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distribution of collagen type 3, % area</td>
<td>0.81±0.07</td>
<td>0.71±0.06</td>
<td>0.81±0.07</td>
<td>0.71±0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distribution of elastin, % area</td>
<td>0.40±0.11</td>
<td>0.26±0.10</td>
<td>0.31±0.08</td>
<td>0.31±0.06</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are given as mean±SD.

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Figure 4. Examples of variation seen in PA elastin fragmentation varying from grade 0 (no fragmentation) to 4 (severe fragmentation). A representative sample from the aorta, which showed grade 0 fragmentation for all patients, is also shown.

Figure 5. Three-dimensional bar chart demonstrates relationship between preoperative PA pressure and PA elastic fiber fragmentation.

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well reflect subtle variations in surgical technique. Figure 2 demonstrates relatively wide variation in dilatation at each time point; this is due in part to the fact that the percent increases are relatively small. Analyses of preoperative, perioperative, and postoperative variables did not demonstrate any factors predictive of root dilatation.

**Morphological Results**

The present results show that there is a major difference between structure in the aorta and the PA in patients who undergo the Ross operation. Although collagen types 1 and 3 were similarly distributed in the aorta and the PA, the aortic media is thicker, and its elastic fibers are more structured. We also demonstrated a wide variation in PA structure within our series of patients who received autografts; they tended to have increases in PA medial thickness, and most strikingly, the elastic fibers had considerable variation in fragmentation. Patients with higher preoperative PA pressures tended to have lower fragmentation scores, suggesting that preoperatively, the PA responds to increased root wall stress by developing more ordered elastic fibers. Longer follow-up will be necessary to determine whether such a mechanism is advantageous and how important a factor is preoperative PA pressure in the determination of whether a pulmonary autograft is the correct valve substitute. In particular, given that elastin fragmenta-

**Mechanical Results**

We demonstrated that the mechanical behavior of the PA in vitro differs from that of the aorta. On average, the aorta is 30% to 40% stiffer and stronger. We presume the lower portion of the stress-strain curve is due to elastin fibers and that beyond the toe point, there is irreversible damage to the elastin fibers and the characteristics are due to collagen fibers. Lowering the stress and observing whether the strain normalizes at different points of the curve will help determine this; however, limitations in tissue availability prevented this in the present study. If we presume this to be true, then (as illustrated in Figure 7) a systolic blood pressure of 200 mm Hg is more likely to lead to elastic fiber disruption and breakage in the PA wall than the aortic wall. This suggests that postoperative blood pressure control may be critically important in patients who receive pulmonary autografts. In addition, there is indirect evidence, albeit from only 1 patient, of adaptation in the mechanical behavior of the pulmonary autograft.

**Study Limitations**

This study has several limitations. Transthoracic echocardiograms are not the gold standard for aortic root dimensions, but we believe that compared with TEE, the reproducibility figures validate its use in terms of our overall conclusions. In addition, a larger patient series with more extended follow-up may allow us to compare aortic root dimensions more accurately according to the original underlying pathology. It is then possible that different trends may emerge for different underlying causes. With both the morphological and mechanical designs, the numbers were too small to allow us to accurately correlate medial wall thickness, fragmentation, and mechanical behavior with subsequent root dimensions. However, given the small spread of our results up to 4 years, it seems that extended follow-up will be needed in addition to larger numbers of subjects before we will be able to accurately correlate both the histological and mechanical methods with clinical outcomes.

**TABLE 4. Mechanical Properties of Aortic and PA Tissue in Patients Who Underwent Pulmonary Autograft Surgery**

<table>
<thead>
<tr>
<th></th>
<th>Aorta</th>
<th>PA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stiffness modulus 1, mPa</td>
<td>$0.13 \pm 0.04$</td>
<td>$0.08 \pm 0.03$</td>
</tr>
<tr>
<td>Stiffness modulus 2, mPa</td>
<td>$3.40 \pm 1.1$</td>
<td>$2.17 \pm 0.89$</td>
</tr>
<tr>
<td>Maximum tensile strength, mPa</td>
<td>$2.00 \pm 0.62$</td>
<td>$1.32 \pm 0.48$</td>
</tr>
<tr>
<td>Maximum strain, %</td>
<td>$145 \pm 35$</td>
<td>$162 \pm 22$</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD.

**TABLE 5. Reproducibility**

<table>
<thead>
<tr>
<th>Aortic Root Dimension</th>
<th>Reanalyzed, %</th>
<th>TEE Comparison, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annulus</td>
<td>4.6</td>
<td>5.6</td>
</tr>
<tr>
<td>Sinuses</td>
<td>6.3</td>
<td>7.3</td>
</tr>
<tr>
<td>Sinotubular junction</td>
<td>6.1</td>
<td>9.1</td>
</tr>
</tbody>
</table>

Results are presented as coefficients of variability for reanalyzed records (TEE comparisons).
Conclusions
We have shown that in our series of patients who received pulmonary autograft aortic root replacement, there was no significant progressive dilatation of the aortic root and that neoaortic regurgitation was not more than mild at 4 years of follow up. In addition, we have shown that the anatomic structure and mechanical behavior of the PA in vitro differ from those of the aorta in patients who undergo pulmonary autograft surgery. Finally, we demonstrated adaptation of PA mechanical function and 1 possible underlying structural mechanism, elastic fiber fragmentation, in response to PA wall stress. Future studies that correlate histological adaptation and mechanical behavior with longer-term clinical outcome should help further delineate the pattern and possible determinants of pulmonary autograft dilatation.

Acknowledgments
Dr Carr-White is a British Heart Foundation Junior Research Fellow. Dr Yacoub is a British Heart Foundation Professor of Cardiac Surgery.

References
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