SURGERY FOR CONGENITAL HEART DISEASE

REMODELING OF THE PORCINE PULMONARY AUTOGRAFT WALL IN THE AORTIC POSITION

Paul H. Schoof, MD^a
Adriana C. Gittenberger-de Groot, MD,
PhD^b
Emile de Heer, PhD^c
Jan A. Bruijn, MD, PhD^c
Mark G. Hazekamp, MD, PhD^a
Hans A. Huysmans, MD, PhD^a

Objective: Dilatation and valve regurgitation are disturbing sequelae of the pulmonary root functioning at systemic pressures. We tried to characterize the histologic mode of adaptation of the neo-aortic wall.

Methods: We compared routine histologic studies, immunohistochemical staining, and computer-assisted morphometric analysis of aortic, pulmonary autograft, and native pulmonary wall specimens from pigs in which, as a newborn, a valveless pulmonary autograft had been implanted in the aorta.

Results: Histologic examination of the pulmonary autograft revealed a viable, normally revascularized wall without degenerative phenomena. Smooth muscle cells were enlarged and rearranged. The characteristic "pulmonary" medial elastin lamellar structure was retained, which was confirmed by morphometry. Immunohistochemistry of the autograft revealed relatively strong staining of type III collagen and alpha smooth muscle actin, exclusive staining of basic fibroblast growth factor, and no staining of proliferation markers proliferating cell nuclear antigen and Ki67.

Conclusion: The developing pulmonary autograft in the aortic position becomes normally revascularized, lacks major degenerative phenomena, and retains its own typical pulmonary morphologic features. Remodeling is accomplished by increased extracellular matrix deposition with collagen as an important constituent. The marked expression of growth factors in the autograft suggests the persistence of increased metabolic activity. (*J Thorac Cardiovasc Surg* 2000;120:55-65)

Dilatation of the pulmonary autograft after the Ross operation in adults has become a matter of serious concern.^{1,2} Disproportionate enlargement of the neo-aortic root has been documented in children after the Ross operation³ and after the arterial switch operation and has been related to neo-aortic valve regurgitation.⁴ Additionally, pulmonary valve regurgitation is commonly found in the pulmonary root at systemic pressures after the Norwood and Damus-Kaye-Stansel operations.^{5,6}

From the Departments of Cardiothoracic Surgery,^a Anatomy,^b and Pathology,^c Leiden University Medical Center, The Netherlands.

Received for publication Jan 7, 2000; revisions requested Feb 4, 2000; revisions received Feb 21, 2000; accepted for publication Feb 28, 2000.

Address for reprints: P. H. Schoof, MD, Department of Cardiothoracic Surgery, D6-50, Leiden University Medical Center, PO Box 9600, 2300 RC Leiden, The Netherlands (E-mail: PSchoof@thorax.AZL.NL).

Copyright © 2000 by The American Association for Thoracic Surgery
0022-5223/2000 \$12.00 + 0 12/1/106970
doi:10.1067/mtc.2000.106970

These observations have excited the interest about how histologic adaptation of the pulmonary root to systemic pressures is accomplished. Clarification of this issue may help to understand late neo-aortic valve failures. This was the aim of our study. We focused on the pulmonary arterial wall, assuming that pulmonary (neo-aortic) root dilatation is a major determinant of neo-aortic valve regurgitation. For this purpose, we characterized the adaptive histologic responses in vascular wall explants of mature pigs in which, as newborns, a valveless pulmonary autograft had been implanted in the aorta.

Methods

Animal model. Five Dutch Landrace piglets (age, 2-3 weeks; weight range, 9.3 ± 0.7 kg) underwent the operation. All animals received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals" as published by the National Academy Press, 1996. The study protocol was approved by the Animal Experimentation Committee of the Leiden University.

Fig 1. Transverse sections of mature wall of native aorta (*left*) and pulmonary autograft (*right*). Note the relatively thin wall and large diameter of the autograft.

Surgery. The operation has been detailed previously.⁷ The (valveless) pulmonary artery main stem was excised and transplanted to the transected ascending aorta. Before reconstruction, transverse segments of approximately 5-mm height of both pulmonary and aortic wall were excised and fixed in paraformaldehyde (3.6%) at room temperature. Pulmonary artery continuity was reestablished by end-to-end anastomosis.

The animals were allowed to thrive until maturity and were killed 10 months after the operation. At autopsy, transverse segments of the proximal great vessels that contained the pulmonary autograft-to-aorta transition area and the remaining native pulmonary artery, distal from the anastomosis, were harvested.

Tissue preparation. Operative specimens were fixed in paraformaldehyde, embedded in paraffin, and stained with hematoxylin-eosin and elastin-van Gieson stains. Autopsy specimens of pulmonary autograft-to-aorta transition area and native pulmonary artery were divided into 2 segments. One part was frozen for immunohistochemical studies, and the other part was fixed in paraformaldehyde for hematoxylin-eosin and van Gieson stains. Sections were made longitudinally to allow the assessment of the pulmonary autograft-to-aorta anastomotic area.

Light microscopy. Hematoxylin-eosin and elastin-van Gieson stains were applied to paraffin sections for routine histologic assessment.

Immunohistochemistry. Frozen tissue sections (4 μ m) were fixed with acetone and incubated for 1 hour with goat-anti-type I, III, IV, and VI collagen (Harlan Sera-lab, Sussex, England), goat-anti-fibronectin and mouse-anti-alpha-smooth muscle actin (Sigma, St Louis, Mo), rabbit-anti-EHS laminin (E-Y Labs, San Mateo, Calif), rabbit-anti-factor VIII and mouse-anti-proliferating cell nuclear antigen (PCNA; DAKO, Copenhagen, Denmark), mouse-anti-basic fibroblast growth factor (bFGF; Upstate

Biotechnology, Lake Placid, NY), mouse-anti-Ki67 (Immunotech, Marseille, France), and rabbit-anti-transforming growth factor- β_1 (TGF- β_1). Secondary incubation was performed with the horseradish peroxidase-conjugated antibodies: rabbit-anti-mouse immunoglobulin, rabbit-anti-goat immunoglobulin, or swine-anti-rabbit immunoglobulin (DAKO). Diaminobenzidine-developed slides were counterstained with hematoxylin. Light microscopic findings were semiquantitatively scored by 2 investigators.

Morphometry. Medial elastin density, fiber number, and mean size of the nonfiber area were determined by computer-assisted image analysis on a Zeissvision KS400 system (Zeiss, Göttingen, Germany). A software program was developed by us to select elastin fibers by color, intensity, and saturation of the signal. Elastin density was measured as a percentage of the total field area. Fiber numbers were counted after they were digitally skeletonized, and mean nonfiber area was determined as the mean area of gaps between elastin fibers in van Gieson-stained sections by random selection of 5 circular fields within the media of each vessel wall specimen.

Statistics. The average elastin density, fiber count, and mean nonfiber area were compared between aorta, pulmonary autograft, and native pulmonary wall specimens with analysis of variance. Analysis was performed with SPSS version 9 (SPSS, Inc, Chicago, Ill).

Results

All animals were healthy and had attained a 19.4-fold mean increase of body weight (mean $\pm$ SD, 179.2 $\pm$ 10.4 kg; range, 161 to 186 kg) when put to death.

Gross inspection. Growth of the pulmonary autograft paralleled the anatomic development of the animal.⁷ The diameter of the vessel lumen was slightly

Fig 2. Longitudinal microscopic section of pulmonary autograft to aorta transition area (*top*). (Elastin–van Gieson stain; original magnification, ×5). Scar tissue is covered by neointima. The aortic media (*bottom right*) is thicker and shows more numerous and organized elastin layers on detail (original magnification, ×100) than does the pulmonary autograft (*bottom left*; original magnification, ×100.)

Fig 3. For legend see opposite page.

Table I. Light microscopic scores of immunohistochemical stainings in vascular wall specimens

	Aorta	Pulmonary autograft	Native pulmonary artery	Anastomotic scar
Collagen I	± ^{2,3}	+ ^{2,3}	+ ^{2,3}	+
Collagen III	+ + ^{2,3}	+ + + ^{2,3}	+ + ^{2,3}	+ + +
Collagen IV	± ¹	± ¹	± ¹	+
Collagen VI	± ¹	± ¹	± ¹	± ¹
Laminin	± ¹	± ¹	± ¹	± ¹
Fibronectin	+ + ^{2,3}	+ + ^{2,3}	+ + ^{2,3}	+ + +
Factor VIII	+ + ¹	+ + ¹	+ + ¹	+ + ¹
α-SM actin	+ + ²	+ + + ^{2,3}	+ + ²	—
TGF-β	±	+ ^{2,3}	—	—
bFGF	—	+ ²	—	±
PCNA	—	—	—	±
Ki67	—	—	—	±

Expression of intensity was scored from + + + (strong) to ± (faint) to — (absence). Wall segments are numbered as follows: 1 = endothelium of main wall segment or vasa vasorum; 2 = media; 3 = adventitia. α-SM actin, α-smooth muscle actin; TGF-β, transforming growth factor-β; bFGF, basic fibroblast growth factor; PCNA, proliferating cell nuclear antigen; Ki67, proliferation antigen Ki67.

larger than that of the aorta in 3 of 5 specimens. There was no anastomotic narrowing; the luminal aspect of the autograft wall looked healthy, and the wall felt thinner and more pliable than the aortic wall. On transverse sections, the autograft exhibited less wall thickness than the aorta (Fig 1).

Hematoxylin-eosin and elastin stains. The intima showed an intact endothelial layer across the anastomosis between the pulmonary autograft and native aorta, with focal thickening only over the scar.

The medial elastin lamellar structure of the autograft resembled that of the native pulmonary artery, although elastin lamellae of the autograft appeared to be arranged in a more orderly fashion. Additionally, smooth muscle cells in the autograft media were oriented more longitudinally.

Contrary to the autograft media, the aortic media was thicker with more numerous elastin lamellae well-organized in concentric layers (Fig 2).

Fragmentation of elastin lamellae or areas of necrosis were not seen in the autograft wall.

The adventitia of the autograft showed the presence of normal vasa vasorum and appeared to stain more densely in hematoxylin-eosin sections than in native pulmonary artery.

The anastomotic scar between the autograft and aorta showed large numbers of vasa vasorum and was virtually devoid of elastin.

Immunohistochemistry. The results of staining scores are summarized in Table I. Among the tested collagen antibodies, the expression of collagen I was slightly more in the autograft than in the aorta but was not different from that of native pulmonary artery. Collagen III, which was strongly expressed in the anastomotic scar, also showed a relatively strong expression in the autograft. In the media, expression was confined to interlamellar spaces, whereas in the adventitia the staining was more diffuse (Fig 3). The expression of other collagen types (IV and VI) was generally restricted to endothelial cells of the vascular luminal side and vasa vasorum of the various wall specimens. Laminin was faintly stained between medial smooth muscle cells and in scar tissue and more pronounced in basal lamina. Fibronectin was strongly expressed in the anastomotic scar and moderately expressed in both media and adventitia of all specimens. Factor VIII exclusively stained vasa vasorum within the vascular wall. Without background staining, the number and distribution of vasa vasorum in the autograft were shown to be similar to those in the native pulmonary artery (Fig 3). Alpha smooth muscle actin expression seemed particularly strong in the autograft and apparently related to medial smooth muscle cells with large nuclei. It was absent in the anastomotic scar. The pattern of expression was generally coarse in the autograft and native pulmonary

Fig 3. Micrographs of pulmonary autograft show immunohistochemical localization of collagen III (*top*): high density, particularly in the adventitia. bFGF (*center three panels*): large nuclei of autograft smooth muscle cells. *Middle* (original magnification, ×100) and *left panels* (original magnification, ×200) exhibit a peripheral dark shade that is absent in the native pulmonary artery (*right*; original magnification, ×100). Factor VIII (*bottom*): vasa vasorum in autograft adventitia and outer media.

Fig 4. Micrographs of pulmonary autograft (*top*) and native aortic media (*bottom*) show immunohistochemical localization of α -smooth muscle actin (original magnification, $\times 100$); a coarse pattern of expression particularly related to large nuclei in the autograft compared with the native aorta.

artery, whereas in the aorta it had a much finer structure (Fig 4). Compared with the native pulmonary artery, the autograft exhibited more numerous large smooth muscle cells with large nuclei that were frequently arranged in longitudinal fashion, which indicated a change of phenotype. Fibronectin was particularly strongly expressed in the anastomotic scar tissue

and equally moderate in the media and adventitia of all specimens. TGF- β , although faintly expressed in the inner one third of the native pulmonary and aortic media, was more strongly expressed throughout the media of the autograft, with focal condensations at the adventitial-medial interface. A patchy staining pattern was found in the scar. bFGF was exclusively expressed

Fig 5. Results of morphometric analysis of elastin density in the aortic wall (a) and native pulmonary artery (b). At operation (T_0), no difference between aortic and pulmonary density (a vs b ; $P = .25$). At autopsy (T_1), there was a significant difference between native aortic and pulmonary wall (* a' vs b' ; $P = .007$) and native aortic wall and autograft (** a' vs c ; $P = .018$), whereas no difference was observed between the native pulmonary artery and the autograft (b' vs c ; $P = .63$).

in the autograft and was confined to smooth muscle cells between the elastic lamellae (Fig 3). A patchy staining pattern was present in the anastomotic scar.

Proliferation markers PCNA and Ki67, indicators of active cellular replication, were not expressed in any of the vascular specimens and only faintly stained the scar tissue.

Morphometry. Medial elastin density in the aortic wall of newborn pigs did not differ from the pulmonary wall ($P = .25$). By 10 months of age, in the adult pig, a significant difference in elastin density had evolved between the native aortic and pulmonary walls ($P = .007$) and the autograft wall ($P = .018$), whereas no difference could be demonstrated between the native pulmonary artery and the autograft ($P = .63$; Fig 5).

The mean number of elastin fiber fragments in the newborn aortic wall did not differ from the pulmonary wall ($P = .31$) but became significantly less after growth in the aorta compared with the pulmonary artery ($P = .030$) and compared with the autograft ($P = .012$), whereas no difference was found between the native pulmonary artery and the autograft ($P = .64$; Fig 6). This may seem contradictory because the total number of elastin lamellae in the normal aortic wall is, after all, higher than in the normal pulmonary wall. These measurements, however, represent the regularity of the digitally skeletonized elastin fibers, which is

Fig 6. Results of morphometric analysis of the number of elastin fiber fragments. The mean number was not different in wall segments of newborns (a vs b ; $P = .31$), whereas at autopsy it was significantly less in the aorta than in the pulmonary artery (* a' vs b' ; $P = .030$) and in the aorta than in the autograft (** a' vs c ; $P = .012$). No difference was observed between the autograft and the native pulmonary artery (b' vs c ; $P = .64$).

of higher order in the aorta. Therefore, fewer fiber fragments were counted in single fields in the aortic media.

The mean size of the nonfiber area in the wall segments of newborns did not differ ($P = .23$). After growth, it became significantly larger in the aorta compared with the pulmonary artery ($P = .032$) and the autograft ($P = .021$), whereas there was no difference between the autograft and the native pulmonary artery ($P = .81$; Fig 7).

Aortic and pulmonary wall segments apparently developed their own typical medial structure, despite the transplantation.

Discussion

Basic morphologic features. We were surprised to find that, by light microscopy in the autograft, the medial elastin lamellar pattern of the pulmonary wall had been preserved. Maintenance and development of an aortic type of medial pattern would have seemed more appropriate and in accordance with older literature⁸; however, despite early transplantation and development at systemic pressures, the basic morphologic condition of the pulmonary autograft was still characteristically “pulmonary.” We considered this to be an important finding and therefore extended our study by performing morphometric measurements to see whether these light microscopic findings could be sub-

Fig 7. Results of morphometric analysis of the nonfiber area. The mean size of the nonfiber area was not different in wall segments of newborns ($P = .23$), whereas at autopsy it was significantly larger in the autograft (* a' vs c ; $P = .021$) and in the native pulmonary artery (a' vs b' ; $P = .032$) than in the aorta. No difference was found between the autograft and the native pulmonary artery (b' vs c ; $P = .81$).

stantiated. These results confirmed our impression. The characteristic medial pattern of the pulmonary wall is apparently not altered by transplantation to the aortic position and may be genetically predetermined, which would be in accordance with experimental data on the developmental accumulation of vascular elastin, which suggests that factors other than blood pressure contribute to determine vascular morphologic features.^{9,10} Genetic information on dimensional position of the vessel within the body and embryologic origin of vessel wall smooth muscle cells seem to play a critical role in this process.^{11,12}

Consequently, the normal quantitative relationship that exists between vascular structure and mechanical properties according to Wolinsky and Glagov¹³ and Glagov and colleagues¹⁴ would not be applicable in the pulmonary autograft in the aortic position because the number of elastin lamellae is not in proportion to the pressure that is distending the wall. When the autograft does not acquire the architecture of the aortic wall, normal systemic blood pressures could be considered an unphysiologic mechanical load for the pulmonary autograft. Adaptation of the pulmonary wall to systemic hemodynamics is apparently accomplished in a different way.

Remodeling. A vascular wall adapts to excessive mechanical stress by the reorganization of wall constituents to maintain wall integrity and restore baseline conditions of wall stress.¹⁵ The mode of this remodeling

is well known in systemic arteries, pulmonary arteries, and veins at hypertension and is primarily characterized by an increased matrix deposition that results in a thicker wall.¹⁵⁻¹⁷ The features of remodeling that we observed in the pulmonary autograft were similar. However, macroscopically, adaptation did not result in a wall thickness that matched the thickness of the aortic wall. This is not in accordance with the principle that vascular walls, subject to hypertension, thicken until normal wall stress is reestablished (radius/thickness ratio conservation).¹⁸ Additionally, the autograft had acquired a relatively large circumference in 3 of 5 specimens, which suggested dilatation (Fig 1).

At microscopic examination, the autograft was found to be viable and revascularized without degenerative phenomena in the wall segment. Autocrine growth factors TGF- β and bFGF, which are normally not or only faintly expressed in mature quiescent arterial wall,¹⁹ were found to be expressed in the pulmonary autograft wall, which indicated growth activity. As potent stimulators of smooth muscle cell growth and differentiation,^{19,20} both these factors are probably responsible for the observed change in muscle cell phenotype and increased alpha smooth muscle actin immunoreactivity of the autograft. The observed enlargement and rearrangement may indicate an acquired synthetic phenotype of smooth muscle cells that are stimulated to produce extracellular matrix proteins, such as collagen and elastin. Because collagen III reactivity seemed to be strongest in the autograft, this may be an important matrix constituent that is involved in pulmonary autograft remodeling. It seems logical because collagen has the highest tensile force of vascular matrix components and therefore seems to be an obvious constituent for fortification of the vessel wall against the mechanical injury of excessive tensile stress.

Although growth factors were determined in the autograft, cell division could not be detected in any of the mature vessel wall specimens with the use of the proliferation markers PCNA and Ki67. Maybe we missed the cellular replication spurt in the autograft if it was confined to the early postoperative period, as in venous grafts in the systemic circulation¹⁸ and should the presence of growth factors be explained by an increased metabolic demand. This may be caused by increased matrix turnover in the autograft wall and interpreted as an ongoing activity to re-establish wall stress homeostasis.

Dilatation. Our findings do not clarify the propensity of the pulmonary root to dilate at systemic pressures. Nevertheless, dilatation of the unsupported pulmonary autograft seems conceivable when the low elastic modulus of the adult pulmonary wall is considered (one fifth

of that of the aortic wall)²¹ and when the much higher tensile stress of systemic blood pressure is exerted on the neo-aortic wall (8- to 10-fold, according to the LaPlace law). Furthermore, the tendency to dilate was shown both clinically after pulmonary artery banding and in *in vitro* experiments in porcine pulmonary roots.²²⁻²⁴

Our findings suggest that dilatation is not compensated for by a significant increase in wall thickness, although it would be expected considering the principle of r/t ratio conservation.¹⁸ Instead, it seems that collagen is deposited to bear the increased mechanical load. Fortifying the wall in this way will probably also make it stiffer. This occurs in a wall that is already stiffer than the normal pulmonary artery wall because of excessive stretch as the result of systemic blood pressures.²⁵ Theoretically, therefore, systemic pressures may cause loss of distensibility and cause the pulmonary root to become a frozen root. The functional implications of this stiffening,²⁶ in addition to changes in root geometry because of dilatation, may both contribute to early and late neo-aortic valve regurgitation.

Pathologic features. The most important question remains: What will be the long-term consequences of these findings? Should pulmonary wall remodeling be considered as a benign form of scar formation, which, like a dermal scar, will not fail and maintain life-long integrity or should it be viewed as a potential initiation of degenerative vascular disease with a possibility of secondary structural wall failure, as in hypertensive native pulmonary vascular disease?²⁷ Long-term function of the neo-aortic root, however, is probably not determined by wall integrity but rather by the secondary effects of altered dynamic properties and geometry of the neo-aortic root on neo-aortic valve function.

Study limitations. Our findings are based on an animal study. Therefore, the results may not accurately simulate the human process of pulmonary wall remodeling.

Light microscopic findings were derived from longitudinal tissue sections, whereas routine vascular wall sections are usually performed in transverse direction.

The results of the immunohistochemical study were semiquantitatively scored. Quantitative assessment of changes of the various matrix components should be performed to verify our findings.

The documented remodeling response is derived from a pulmonary autograft that was transplanted as a free graft very early in life. The results therefore may not represent the response that may occur later in life nor the remodeling that may occur in the orthotopic pulmonary root at systemic pressures. Furthermore, the study focuses on supracommissural wall specimens.

Therefore, the observed changes may not represent the changes that occur below, in the sinus walls.

Conclusions

The developing pulmonary autograft in the aortic position becomes normally revascularized, lacks major degenerative phenomena, and retains its own typical pulmonary morphologic features. Remodeling is accomplished by the increased deposition of extracellular matrix constituents, with collagen being an important component. The marked expression of growth factors in the autograft suggests the persistence of increased metabolic activity.

We thank the animal laboratory technicians for their skilled assistance during the study, Hans Baelde for assistance on tissue specimen processing, Jan Schuttrups for the morphometry, Klaas van der Ham for the photography, Evelien van Westerop and Piet van Sighem for the secretarial work, and Koos Zwinderman for the statistics.

REFERENCES

1. David TE, Omran A, Ivanov J, Armstrong S, de Sa MPL, Sonnenberg B, et al. Dilation of the pulmonary autograft after the Ross procedure. *J Thorac Cardiovasc Surg* 2000;119:210-20.
2. Elkins RC, Lane MM, McCue C, Ward KE. Pulmonary autograft root replacement: mid-term results. *J Heart Valve Dis* 1999;8:499-506.
3. Elkins RC, Knott-Craig CJ, Ward KE, McCue C, Lane MM. Pulmonary autograft in children: realized growth potential. *Ann Thorac Surg* 1994;57:1387-94.
4. Blume ED, Wernosky G. Long-term results of arterial switch repair of transposition of the great vessels. *Semin Thorac Cardiovasc Surg* 1998;1:129-37.
5. Schmid FX, Hilker M, Kampmann C, Mayer E, Oelert H. Clinical performance of the native pulmonary valve in the systemic circulation. *J Heart Valve Dis* 1998;7:620-5.
6. Hanley FL. Clinical experience with the autologous pulmonary valve in the systemic circulation. *Semin Thorac Cardiovasc Surg* 1994;6:41-7.
7. Schoof PH, Hazekamp MG, van Wermeskerken GK, de Heer E, Bruijn JA, Gittenberger-de Groot AC, et al. Disproportionate enlargement of the pulmonary autograft in the aortic position in the growing pig. *J Thorac Cardiovasc Surg* 1998;115:1264-72.
8. Heath D, Wood E, du Shane J, Edwards J. The structure of the pulmonary trunk at different ages and in cases of pulmonary hypertension and pulmonary stenosis. *J Pathol Bacteriol* 1959;77:443-56.
9. Keeley FW, Rabinovitch M. Vascular matrix metabolism in hypertension. In: Bishop JE, Reeves JT, Laurent GJ, editors. *Pulmonary vascular remodelling*. London: Portland Press Ltd; 1995. p. 150.
10. Bendeck MP, Langille BL. Rapid accumulation of elastin and collagen in the aortas of sheep in the immediate perinatal period. *Circ Res* 1991;69:1165-9.
11. Bergwerff M, de Ruiter MC, Gittenberger-de Groot AC. Comparative anatomy and ontogeny of the ductus arteriosus: a vascular outsider. *Anat Embryol* 1999;200:559-71.

12. Bergwerff M, de Ruiter MC, Poelman RE, Gittenberger-de Groot AC. Onset of elastogenesis and downregulation of smooth muscle actin as distinguishing phenomena in artery differentiation in the chick embryo. *Anat Embryol* 1996;194:545-57.
13. Wolinsky H, Glagov S. A lamellar unit of aortic medial structure and function in mammals. *Circ Res* 1967;20:99-111.
14. Glagov S, Vito R, Giddens DP, Zarins CK. Micro-architecture and composition of artery walls: relationship to location, diameter and the distribution of mechanical stress. *J Hypertens* 1992;10(suppl):S101-4.
15. Gibbons GH, Dzau VJ. The emerging concept of vascular remodeling. *N Engl J Med* 1994;330:20:1431-8.
16. Stenmark K, Mecham RP. Cellular and molecular mechanisms of pulmonary vascular remodeling. *Annu Rev Physiol* 1997;59:89-144.
17. Shi Y, O'Brien JE, Mannion JD, Morrison RC, Chung W, Fard A, et al. Remodeling of autologous saphenous vein grafts. *Circulation* 1997;95:2684-93.
18. Zwolak RM, Adams MC, Clowes AW. Kinetics of vein graft hyperplasia: association with tangential stress. *J Vasc Surg* 1987;5:126-36.
19. Botney MD, Bahadori L, Gold LI. Vascular remodeling in primary pulmonary hypertension: potential role for transforming growth factor- β . *Am J Pathol* 1994;144:286-95.
20. Klagsbrun M, Edelman ER. Biological and biochemical properties of fibroblast growth factors. *Arteriosclerosis* 1989;9:269-78.
21. Milner WR. Hemodynamics. 2nd ed. Baltimore: Williams & Wilkins; 1989. p. 76.
22. Sievers HH, Lange PE, Arensman FW, Radley-Smith R, Yacoub MH, Harms D, et al. Influence of two-stage anatomic correction on size and distensibility of the anatomic pulmonary/functional aortic root in patients with simple transposition of the great arteries. *Circulation* 1984;70:202-8.
23. Sievers HH, Leyh R, Loose R, Guha M, Petry A, Bernhard A. Time course of dimension and function of the autologous pulmonary root in the aortic position. *J Thorac Cardiovasc Surg* 1993;105:775-80.
24. Nagy ZL, Fisher J, Walker PG, Watterson KG. The influence of size mismatch on the hemodynamic performance of the pulmonary autograft in vitro. *Eur J Cardiothorac Surg* 1999;15:294-301.
25. Zuckerman BR, Orton C, Stenmark K, Trapp JA, Murphy JR, Coffeen PR, et al. Alteration of the pulsatile load in the high-altitude calf model of pulmonary hypertension. *J Appl Physiol* 1991;70:859-68.
26. Leyh RG, Schmidtke C, Sievers HH, Yacoub MH. Opening and closing characteristics of the aortic valve after different types of valve-preserving surgery. *Circulation* 1999;100:2153-60.
27. Barter T, Irwin RS, Nash G. Aneurysms of the pulmonary arteries. *Chest* 1988;94:1065-75.

Commentary

Dr Schoof and coauthors are to be complimented on their continuing efforts to unravel the complex adaptive response that occurs when the pulmonary artery and its contained valve are placed in the systemic circulation. This work has significant interest to all cardiac surgeons and cardiologists involved in the care and management of patients who have had surgical procedures

that use the pulmonary artery and valve as the systemic output for the heart. Such procedures include the Ross, arterial switch, Damus-Kaye-Stansel, and Norwood procedures. All of these have been associated with episodes of failure of the valved conduit, usually related to progressive dilatation, loss of adequate leaflet coaptation, and central neo-aortic valve regurgitation. Dr Schoof has used the growing pig as an experimental model of the Ross operation and has provided valuable insight into the growth characteristics associated with translocation of the pulmonary artery and its contained valve into the systemic circulation. Dilatation of this structure is evident, particularly at the point of distention, when the aortic crossclamp is removed and the autograft valve and conduit are subjected to systemic pressures and modulation or remodeling of the valve cusps and of the pulmonary artery. The authors have previously shown significant increase in valve cuspal weight, valve cuspal height, and valve cuspal width after a Ross operation. They have shown an increase in the circumference of the pulmonary autograft wall over a 10-month period in the growing pig, as well as an increase in thickness and volume. These changes in dimensions closely match what one would anticipate in terms of demonstrated clinical performance of the pulmonary autograft when it is used as a root replacement in patients. In the present study, the authors have provided a morphometric analysis of the histology and immunohistochemistry of the autograft root in a growing pig model. They have demonstrated that the adaptive response of the pulmonary autograft is not one of adaptation toward a typical aortic medial pattern but one that retains the basic morphology of the pulmonary root. They found the autograft wall to be viable, revascularized, and without degenerative phenomena. Enlargement and rearrangement of smooth muscle cells and an increase in collagen III were identified.

The recent studies are extremely important and helpful to physicians concerned about the fate of patients requiring use of the pulmonary artery in its contained valve for systemic ventricular outflow. However, the authors raise many unanswered questions that are open for further study and detailed analysis. The authors' data are primarily limited to the pulmonary autograft at the autograft aortic transition area and circumferential segments of the autograft, probably all distal to the sinotubular junction of the autograft. The fate of the autograft sinuses, the thinnest segment of the pulmonary root, is unclear.

These studies, along with other clinical information, suggest that one should not anticipate that the autograft root will behave like an aortic root when transplanted