19 Heart, Heart Valves, and Vessels

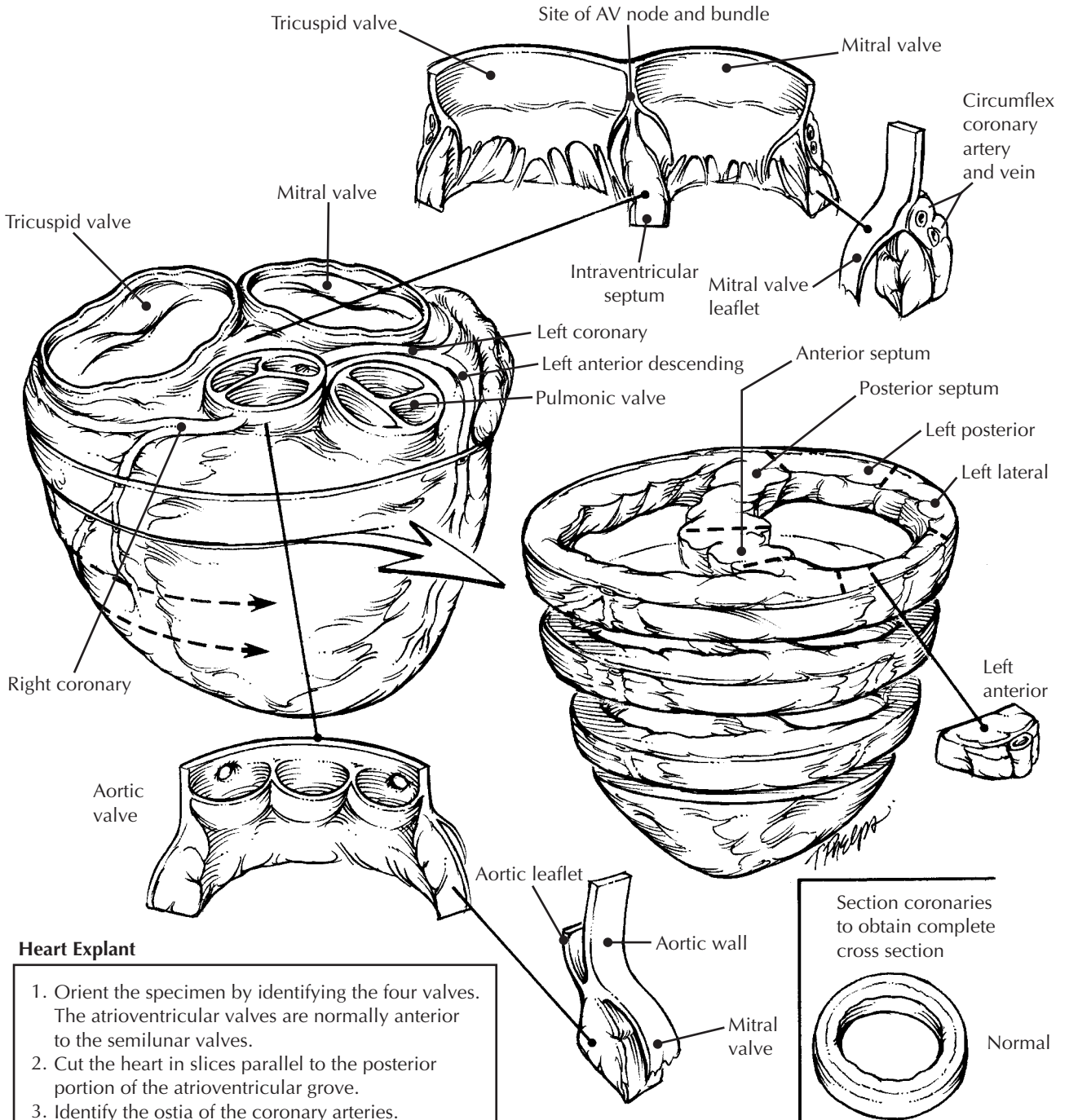
E. Rene Rodriguez, M.D.

The Explanted Heart

The examination of hearts explanted from heart transplant recipients provides a unique opportunity to study cardiac pathology. Moreover, in some instances it influences posttransplant therapy and prognosis.

The specimen should be emptied of clotted blood and weighed before examination. Document the general shape (globular or normal) and consistency (firm or floppy) of the heart, and identify the major structures (ventricles, atria, pulmonary artery, root of the aorta). In most instances the atria are partially or completely missing, and this should be recorded. The pulmonary artery and valve as well as the aortic root and valve may be intact or partially torn during the resection of the heart. Document the anatomy. Do the great vessels emerge from the heart in their normal anatomic location? If you suspect a congenital heart disease, you should work carefully with the clinician and review appropriate preoperative imaging, as the clinical history guides your dissection. Further examination of the heart is simplified if you approach the three layers of the heart (epicardium, myocardium, endocardium), the valves, and the coronary vessels systematically.

Start with the epicardium. Examination of the external surface of the heart should document the presence or absence of petechiae, adhesions (fibrous or fibrinous), scar (focal or extensive), calcification, and grafts (vascular or synthetic material). If grafts are present, document their location and the status of the anastomoses. Grossly examine the valves as best you can before sectioning the heart. Document any thrombi or vegetations. In most instances transverse sectioning of the heart at 1-cm intervals from the apex to just below the atrioventricular apparatuses allows the diagnosis of hypertrophy and/or dilatation, and it demonstrates the greatest surface area of the sectioned myocardium. Examine the myocardium carefully. The size and location of recent or remote infarcts, focal punctate lesions, hemorrhages, fibrosis, or frank necrosis should be noted. Examination of the myocardium should be guided by the patient's clinical history, as some pathologic conditions affect specific areas of the heart. For example, sarcoidosis tends to begin in the basal portion of the heart close to the atrioventricular apparatus; on the other hand, right ventricular dysplasia affects the right ventricular free wall during early stages and spreads to the left ventricle late in the disease. Next, turn your attention to the endocardium and valves. Are the endocardial surfaces smooth, or are focal lesions noted? Examine the atrioventricular and semilunar valves, as described later under Heart Valves. Lastly, the coronary arteries can be examined. Do this systematically. Start at the orifice of the left main coronary artery and serially section the vessel proceeding down the left anterior descending and left circumflex arteries as far as possible. Similarly, section the right coronary artery from its orifice to its distal branches. Document the course of these vessels. Are they in their normal anatomic location? As described in detail under Arteries and Veins, examine the lumen of each vessel for thrombi; examine the intima for evidence of intimal proliferation, hemorrhage, or atheromatous plaques; and document the location and percentage narrowing caused by any lesions. For example, your report could include "there is 90% stenosis of the left anterior descending coronary arteries just proximal to the take-off



Heart Explant

1. Orient the specimen by identifying the four valves. The atrioventricular valves are normally anterior to the semilunar valves.
2. Cut the heart in slices parallel to the posterior portion of the atrioventricular grove.
3. Identify the ostia of the coronary arteries. Serial section each coronary artery from its ostium to its distal branches.
4. Submit sections of any lesions as well as sections of the coronary arteries (in cross section), myocardium (septum, left posterior wall, left lateral wall, and left anterior wall) and of the atrioventricular and semilunar valves.
5. When sampling the atrioventricular valves, also include a small segment of atrial wall and ventricular wall. When sampling the semilunar valves, include a segment of the great vessel (i.e., aorta or pulmonary artery) in your section.

of the first diagonal branch." It is not uncommon to receive specimens with one or more metal stents within the coronaries. If stents are present, describe their presence and location. Section the vessel close to the stent and inspect its lumen. Sectioning through the stents is not feasible in common pathology practice.

The heart can now be sampled. Samples of myocardium for electron microscopy should always be procured. Also in modern pathology practice, pieces of each ventricle should be frozen in optimal controlled temperature (OCT) compound and also snap-frozen for any molecular diagnostic test that may be needed later. Sections submitted in formalin for histopathologic examination should include a minimum of four sections of the ventricles (interventricular septum, anterior wall, lateral wall, and posterior wall); a section of any valve lesions; a section of the mitral and aortic valves; and representative sections of each of the coronary arteries. The section of the anterior wall can often be taken to include the left anterior descending coronary artery. Sample any additional area showing gross pathologic changes. Examination of the atrioventricular conduction system is often possible, whereas in most instances examination of the sinoatrial node is not. It is easy to sample the conduction system if needed (see The Conducting System). Remember that atherosclerosis and some valve lesions can be quite calcified, and therefore decalcification should be performed as necessary.

The Conduction System

Histologic examination of the conduction system is difficult and time-consuming, and it is usually fruitless unless the patient has a clinical history of a complete heart block. Nonetheless, in some instances examination of the conduction system can provide critical insight into the underlying nature of the patient's pathology.

In general, the sinoatrial node is not present in the explanted heart. Examination of the atrioventricular (AV) node was well described by Hutchins:[3] From the right side of the heart, identify the ostium of the coronary sinus, the septal leaflet of the tricuspid valve, the membranous interventricular septum, and a line approximately 2 cm below the insertion of the septal leaflet of the tricuspid valve. Remove the block of tissue contained within these landmarks by cutting into the septum from the right side. This block of tissue can then be serially sectioned for histologic examination.

Endomyocardial Biopsy

Endomyocardial biopsy is still the gold standard for monitoring the allograft. Biopsies are also frequently performed to determine the etiology of heart failure in nontransplanted patients. The tissue is usually procured with a bioptome through either the jugular or the femoral vein. There is evidence that with three pieces only 95% of inflammatory infiltrates are detected. However, if four pieces are examined, up to 98% of infiltrates are detected. The working formulation for heart allograft monitoring therefore recommends examination of at least four pieces of tissue.[4] Documenting the number of biopsy specimens received is therefore important. The specimens are handled differently depending on the timing or the reason for the biopsy.

Following a few simple rules ensures optimal preservation of the tissue for diagnostic analysis. Minor modifications to these rules for specific tests or research protocols can be made without disrupting the work flow in the heart biopsy suite. Some helpful hints include the following.

1. Plan ahead. Take into account that the working formulation recommends "four to six undivided pieces of tissue," "one piece frozen," and "no tissue routinely fixed for electron microscopy." Commonly, the fixative of choice is 10% phosphate-buffered formalin. Alternatively, fixation can be done in glutaraldehyde for microscopy or in other fixatives that preserve antigens for immunohistochemistry studies.
2. The tissue should not be handled with forceps or divided with a scalpel. The tip of an intravenous catheter or syringe needle is usually a good instrument for picking up the biopsy. Squeezing the tissue can produce artifacts that upon microscopic examination render it uninterpretable.
3. The tissue should be fixed immediately in the desired fixative that has been allowed to reach room temperature. Cold fixative enhances contraction band artifacts. The tissue should not be allowed to sit for long periods of time on filter paper, gauze, or any other surface impregnated with saline. Saline is a poor solution

for preserving the morphology of myocardium, as it readily creates artifacts.

4. During the first six weeks after transplantation, at least one piece of tissue should be frozen. The working formulation recommends that the tissue be frozen in OCT compound (Miles Inc., Diagnostics Division, Elkhart, IN, USA).[4] We prefer to freeze the tissue using isopentane, which should be chilled to −20°C in a small 1.8-ml cryogenic vial. The biopsy tissue is then immersed in this prechilled isopentane cryovial, the cap is tightened, and the container is immersed in liquid nitrogen. At this point the tissue can be processed for immunofluorescence or stored at −80°C for future study.

5. In the nontransplanted patient, one or more pieces of tissue can be snap-frozen for special studies (e.g., immunohistochemistry, *in situ* nucleic acid hybridization, polymerase chain reaction).

For transplant biopsies the working formulation[4] recommends: "a minimum of three step levels through the paraffin block with at least three sections of each level." Similar handling is adequate for nontransplant specimens. Slides should be stained routinely with hematoxylin and eosin; additional unstained slides should be obtained for other stains to avoid having to "face" the paraffin block again and thus minimize tissue loss due to technical handling.

For heart transplant biopsies the working formulation does not require routine submission of tissue from cardiac allograft biopsies for electron microscopy. However, for diagnostic "cardiomyopathy work-up" biopsies, it is important to procure at least one specimen and fix it in glutaraldehyde. If the biopsy is received in formalin and there are more than four biopsy pieces, one may be transferred to glutaraldehyde and submitted for electron microscopy. In cases of suspected adriamycin toxicity, consideration should be given to submitting all of the tissue for electron microscopy.

Cardiac Tumors

Resections of cardiac tumors are not common surgical pathology specimens. Despite their infrequency, these specimens are easily tackled using the standard approach to tumor dissection.

Describe the number and sizes of the tissue pieces received and the presence of epicardium, endocardium, or muscle. Document the size of any masses. The color and texture of the mass are also important to note, as they may indicate the predominant presence of fibrous tissue, myxoid stroma, adipose tissue, or muscle. Intracavitary masses can be pedunculated or sessile and should be described accordingly. The resection margin(s) should be inked and sampled, and the status of these margins should be documented in your final report, as it is useful information for the surgeon. As with any tumor, adequate sampling requires representative sections of areas that may show distinct gross features, such as fibrosis, necrosis, hemorrhage, or a recognizable normal structure (e.g., valve, trabeculae). If the mass is large, one cassette for every centimeter of maximal diameter of the tumor should be adequate. A piece of the tumor may be frozen and stored for special studies, and submission of fresh tissue may be indicated if cytogenetic or flow cytometric analysis is to be performed. Sampling the tumor for these analyses should avoid areas of frank necrosis. If the tumor is heavily calcified, it should be handled similar to a bone specimen for the slicing, sampling, and decalcifying procedures.

Pericardium

The pericardium includes the parietal and visceral pericardium. The parietal pericardium consists of the tough fibrocollagenous tissue sac covering the heart. Its inner surface is lined by mesothelial cells. Usually there are very few blood vessels coursing through it. The visceral pericardium is a more delicate, thinner fibrous layer covering the heart and epicardial fat. In general, the pericardial specimens submitted to surgical pathology are samples of the parietal pericardium, which can become very thick as a result of inflammatory and/or neoplastic infiltration.

In addition to the overall dimension of the piece of tissue received, it is important to record the average thickness of the pericardial sample. Document the presence or absence of adipose tissue, areas of hemorrhage, nodules, and the status of the surface (e.g., smooth, shiny, ragged, fibrinous, granular). Note whether there are fibrin deposits, fibrous collagenous bands, cystic spaces, or papillary projections. Rarely, frank abscesses are demonstrated on gross examination.

Adequate sampling may vary according to the size of the specimen and the clinical information. Careful gross examination aids in determining what should be sampled for histology. A thickened pericardial sample (i.e., pericardial thickness of more than 3 mm) should be fixed and cut perpendicular to the inner surface of the pericardial sac, which in most cases is easily identified. Serial slices may be submitted to maximize the surface area. If the pericardial sample is thin (less than 2 mm in thickness) one should make sure that the sections are embedded "on edge" to perform an adequate histologic examination.

Heart Valves

For years, almost all valvular heart disease has been ascribed to chronic rheumatic heart disease. As a result, excised heart valves are among the most neglected specimens in the surgical pathology laboratory. Failure to pay appropriate attention to resected valves can be a disservice to the patient. Careful examination of heart valves not only will help in the clinical management of these patients but may also help in the development of improved prosthetic heart valves.

As is true for any specimen, clinical information is essential for the appropriate classification of heart valve disease. The results of echocardiography and cardiac catheterization, as well as the surgeon's operative findings, should all be obtained before you begin your examination. Although there is some overlap, the examination of native, mechanical, and bioprosthetic heart valves each presents unique challenges.

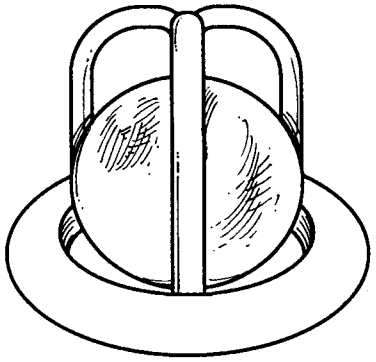
Native Heart Valves

Although we tend to think of heart valves as consisting only of the valve leaflets, remember the other components of the valve. The atrioventricular valves (mitral and tricuspid) are composed of an annulus, leaflets, chordae tendineae, and papillary muscles. The semilunar valves (the aortic and pulmonic) are made up of three cusps, each with a sinus. These cusps meet at three commissures. Remembering these basic components of each valve is essential, because, although some native heart valves can be removed intact by the surgeon, the majority are fragmented during removal.

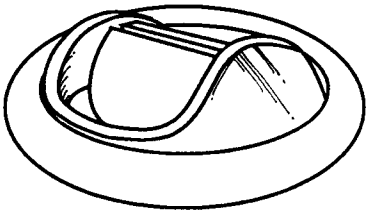
Before handling an excised valve, check to see if cultures are needed. In addition, a photograph of the inflow and outflow aspects of a valve can help document the cause of the disease. Similarly, a radiograph of the specimen may help establish the extent and distribution of calcifications. Begin by documenting whether the valve is in one piece or is fragmented. Note the dimensions of the valve as well as the dimensions of the valve orifice. Next, systematically examine each component of the valve. Start with the leaflets. Count and record the number of leaflets. Note the edges of the leaflets, and look for any evidence of rolling or commissural fusion. Next, examine the leaflets themselves. Document the presence or absence of myxoid changes, fibrosis, calcifications, thrombi, and vegetations. If the leaflets are fibrotic, are they diffusely or focally involved? If calcifications or thrombi are present, document their location, size, and apparent impact on valve function. If vegetations are noted, are they friable or firm? Next, for atrioventricular valves, examine the chordae tendineae and papillary muscles. Are the chordae normal, or are they shortened, thickened, stretched, fused, or ruptured? Are the papillary muscles normal, or is there evidence of recent or remote myocardial infarction? If a portion of the annulus is present, examine it as well. Once you have completed your gross description, submit a section for histology. The section should include the valve leaflet, the free edge of the valve, and if present the chordae and papillary muscle. It may be necessary to decalcify this section.

Mechanical Heart Valves

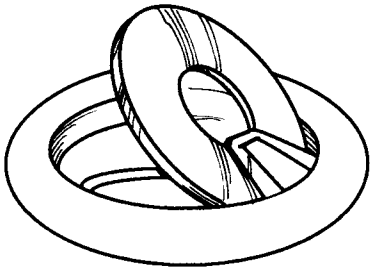
Numerous different types of mechanical heart valves have been developed over the years. The majority of these valves have three main components (1) a cloth ring, which is used by the surgeon to sew the valve in place; (2) an occluder or poppet (ball or disk); and (3) components that limit the movement of the poppet or allow the occluder to tilt. Despite the similarities, the various types of mechanical heart valves have several important differences, and different valves are prone to different complications. Therefore, before beginning your examination of a mechanical heart valve, we recommend that you identify the type of valve by referring to a valve identification guide such as ''Cardiac Valve Identification



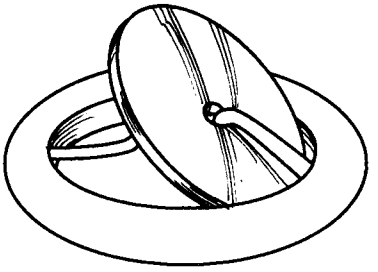
Starr-Edwards
(Ball-in-cage mechanical)



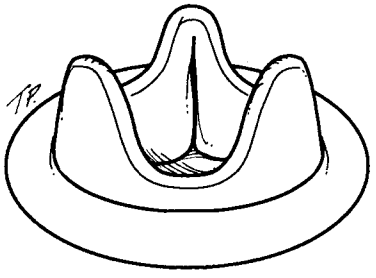
St. Jude
(Bileaflet mechanical)



Bjork-Shiley
(Monoleaflet mechanical)



Medtronic-Hall
(Monoleaflet mechanical)



Carpentier-Edwards and
Hancock porcine
(Tissue valves)

Common Prosthetic Cardiac Valves

1. Identify the type of valve.
2. Look for, document the presence of, and sample for histology any thrombi, vegetations, or fibrous tissue proliferations.
3. Document the movement of the valve.
4. Examine and document the condition of each component of the valve: (1) the valve ring, (2) the occluder or poppet, and (3) components that limit the movement of the poppet or allow the occluder to tilt.
5. *Save the valve.* It's the law.

Atlas and Guide,'' in *Guide to Prosthetic Cardiac Valves*.[5] Examples of some of the more common types of mechanical and bioprosthetic valves are illustrated here.

Next, the valve can be cultured, photographed, and x-rayed as indicated. Try out the movement of the valve. Does the valve apparatus open and close fully? Thrombi can form at the junction of the various components of the valve, especially at cloth-metal interfaces. These thrombi are important to identify, because they can be a source of emboli or a nidus for the development of an infection, and they may interfere with valve function. Document the location and size of any thrombi or vegetations, and note whether they interfere with valve function. Some valves incite an intense overgrowth of fibrous tissue, and this fibrous tissue can cause valve dysfunction and even luminal stenosis. Therefore, it is important to note the presence or absence of fibrous tissue. Finally, carefully look for evidence of wear and tear on the various mechanical components of the valve. Is there any evidence of cracking or disk wear? As is true for natural heart valves, the effect of any pathology on valve function should be carefully documented. In most cases, it is not possible to submit any tissue for histologic examination; but if vegetations are present, a section should be submitted for histology. In the United States, the U.S. Safe Medical Devices Act of 1990 (Public Law 101-629) requires you to notify either the manufacturer or the Food and Drug Administration if you discover that a malfunctioning prosthetic valve has contributed to the harm or death of a patient. Finally, as is true for all mechanical devices removed surgically, be sure to save the valve because it may need to be returned to the manufacturer.

Bioprosthetic Heart Valves

A variety of bioprosthetic heart valves are used. These include (1) porcine aortic valves, (2) bovine and pericardial valves, and (3) human aortic homographs. As is true for the native and mechanical heart valves, the first step should be to ask yourself if the valve needs to be cultured, photographed, or x-rayed. Next, carefully inspect the valve leaflets for thrombi, vegetations, calcifications, and evidence of fibrous overgrowth. In particular, look for evidence of tears or perforations in the valve leaflets. Again, make sure to document both the location and size of any lesions and to describe the effect of these lesions on valve function. If a sewing ring is present, be sure to examine it as well.

Left Ventricular Assist Devices

Examination of left ventricular assist devices requires special tools that may need to be provided by the manufacturer. In general, a pump is connected to conduits that contain artificial valves (usually bioprostheses), which in turn connect to the left ventricular apex and the aorta. These devices can become infected, and it is the responsibility of the pathologist to make every attempt to document any sites of infection. One should document the presence or absence of thrombi or vegetations in the diverse parts in contact with blood. The anastomotic sites to the left ventricle and the aorta should be described and sampled for histologic examination. As is true for any prosthetic device, document any identifying numbers or labels and place the device in a "permanent save" area.

Pacemakers and Defibrillators

Recording the serial number of the pacemaker or defibrillator is the first step. These numbers are usually easy to find when the fibrous sac of tissue surrounding the device is incised. On gross examination, the important things to document are the extent of adhesions on the leads and the location of the active interface of these devices (i.e., the electrode tips) within the heart. Rarely, these devices show infected vegetations. Submit representative sections of any adherent fibrous tissue. Again, as was true for left ventricular assist devices, the device should be placed in a "permanent save" area.

Important Issues to Address in Your Surgical Pathology Report on Heart Valves

Native Heart Valves

- How many leaflets are present?
- Do the leaflets show evidence of fusion, myxoid change, fibrosis, calcification, thrombi, or vegetations?

- Do the chordae show evidence of shortening, thickening, stretching, fusion, or rupture?
- Are the papillary muscles infarcted?
- Do any of these changes appear to have an impact on valve function?

Mechanical and Bioprosthetic Valves

- What type of valve is it?
- Is there evidence of thrombi, fibrous tissue overgrowth, wear and tear, or mechanical failure?
- Is the valve function compromised?

Arteries and Veins

The examination of arteries and veins is straightforward, particularly if one remembers the three layers of each: the intima, media, and adventitia. Measure the length and external and internal diameters of the vessel. Examine the lumen for thrombi, examine the intima for evidence of intimal proliferations such as atherosclerosis, and document the percentage of luminal narrowing caused by any lesions. Examine the media for evidence of aneurysm formation or fibromuscular hyperplasia. Take sections that are both longitudinal and perpendicular to the long axis of the vessel. The longitudinal sections will be particularly helpful in demonstrating alterations involving the media, and the perpendicular sections can demonstrate the effect of any lesion on the luminal diameter.

Temporal Artery Biopsies

Biopsies are occasionally taken of the temporal artery in cases for which temporal arteritis is suspected. These biopsies need to be carefully and thoroughly examined, at multiple levels, for focal disease. The average biopsy is about 12 mm long. Cut it into four pieces, each about 3 mm long, and have the laboratory embed each piece on end. We like to get four sets of step sections through the block, each set containing three hematoxylin and eosin stained sections, one elastin stained section (Verhoeff's/van Gieson's), and one unstained section. Thus, one temporal artery biopsy produces at least 16 slides to examine.