Pathology of the Conduction System in Cardiac Rejection

By CHARLES P. BIEBER, M.D., EDWARD B. STINSON, M.D., AND NORMAN E. SHUMWAY, M.D.

SUMMARY
Correlation of electrocardiographic abnormalities occurring postoperatively with lesions of the conduction system found at autopsy was done in 20 dogs which had received cardiac homografts. The incidence of arrhythmias closely paralleled the severity of rejection injury found within the heart and specifically within the conduction system. Severe pathological changes in the conduction system (grade D) were uniformly associated with arrhythmias. These disturbances, as well as decrease in electrocardiographic voltage, have consistent anatomic correlates in acute rejection of cardiac homografts and are of primary importance in the clinical diagnosis of acute rejection.

Additional Indexing Words:
Heart, transplantation Heart, conduction system Arrhythmias

The electrocardiographic features associated with rejection of cardiac homografts in dogs have been previously described.1 Recent clinical experience with cardiac transplantation has confirmed that the electrocardiogram is a sensitive index of organ dysfunction due to the immune process.2 Abnormalities in human recipients have included diminished electrocardiographic voltage, conduction disturbances, and atrial arrhythmias. These findings suggest early involvement of the cardiac conduction system in rejection injury and form the basis for the following report.

Methods
Cardiac homografts were placed in 20 dogs and autografts in five, by methods previously described.3, 4 Postoperative atrial pacing was used when required. Digitalis was not given to any of the animals.
Three-lead electrocardiograms were taken from the first postoperative day until death. R-wave voltage in lead II was selected for measurement, and most electrocardiographic changes occurring after the third postoperative day were considered to be manifestations of rejection injury.
No uniform effort was made to obtain prolonged survival, although all dogs received some immunosuppressive therapy. Twelve dogs were given azathioprine, 50 mg daily beginning on the day of operation, and nine of these dogs

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Circulation, Volume XXXIX, May 1969 567
Table 1

<table>
<thead>
<tr>
<th>Dog</th>
<th>Survival time (days)</th>
<th>Treatment</th>
<th>Cause of death</th>
<th>Acute rejection (grade)</th>
<th>ECG</th>
<th>Voltage</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA9</td>
<td>3</td>
<td>Azathioprine</td>
<td>Acute rejection</td>
<td>I</td>
<td>B</td>
<td>Not assessed</td>
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<tr>
<td>LA5</td>
<td>4</td>
<td>ALS†</td>
<td>Acute rejection</td>
<td>II</td>
<td>B</td>
<td>None</td>
</tr>
<tr>
<td>LA2</td>
<td>4</td>
<td>ALS</td>
<td>Acute rejection</td>
<td>II</td>
<td>B</td>
<td>None</td>
</tr>
<tr>
<td>LA19</td>
<td>5</td>
<td>Azathioprine</td>
<td>Acute rejection</td>
<td>III</td>
<td>C</td>
<td>None</td>
</tr>
<tr>
<td>LA8</td>
<td>5</td>
<td>ALS Prednisolone</td>
<td>Acute rejection</td>
<td>III</td>
<td>D</td>
<td>Intraventricular block</td>
</tr>
<tr>
<td>LA20</td>
<td>5</td>
<td>Azathioprine Prednisolone</td>
<td>Acute rejection</td>
<td>IV</td>
<td>B</td>
<td>None</td>
</tr>
<tr>
<td>LA16</td>
<td>5</td>
<td>ALS</td>
<td>Acute rejection</td>
<td>III</td>
<td>B</td>
<td>None</td>
</tr>
<tr>
<td>LA4</td>
<td>6</td>
<td>ALS</td>
<td>Acute rejection</td>
<td>II</td>
<td>C</td>
<td>None</td>
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<tr>
<td>LA10</td>
<td>8</td>
<td>Azathioprine Prednisolone</td>
<td>Acute rejection</td>
<td>IV</td>
<td>D</td>
<td>Atrial flutter</td>
</tr>
<tr>
<td>LA18</td>
<td>18</td>
<td>ALS</td>
<td>Acute rejection</td>
<td>III</td>
<td>C</td>
<td>Nodal rhythm</td>
</tr>
<tr>
<td>LA7</td>
<td>19</td>
<td>Azathioprine</td>
<td>Acute rejection</td>
<td>IV</td>
<td>D</td>
<td>First and second degree block</td>
</tr>
<tr>
<td>LA17</td>
<td>26</td>
<td>Azathioprine Prednisolone</td>
<td>Technical</td>
<td>IV</td>
<td>D</td>
<td>Sinus arrest Atrial flutter</td>
</tr>
</tbody>
</table>

* Conduction system.
† Antilymphocytic serum.

were subsequently treated additionally with methylprednisolone for rejection. Eight dogs received antilymphocytic serum (leuko-agglutination titer 1/256), 1 ml/kg daily subcutaneously, starting at the time of surgery, and two of these were subsequently treated with methylprednisolone as well.

All dogs were autopsied and the immediate cause of death was established in all but one animal (tables 1 to 3). Seventeen of the experimental hearts and all of the control hearts were opened along the lines of blood flow and examined grossly with particular reference to the blood supply of the sinus and atroventricular (A-V) nodes. After formalin fixation, blocks of tissue containing the sinus node and A-V system were removed and sectioned subserially. The remaining three experimental hearts were packed with cotton and fixed in formalin. After fixation subserial 5-μ frontol sections were made through the entire heart. Sections from all hearts were stained with hematoxylin and eosin, and selected sections were stained with Masson trichome, phosphotungstic acid-hematoxylin, periodic acid-Schiff reagent, and methyl green-pyronine.

**Histological Assessment of Rejection**

Microscopic changes in the orthotopically homografted canine hearts were similar to those reported by Kosek and associates. In assessing the stage of rejection we first attempted to define and exclude (1) ischemic changes due to surgical manipulation of the graft's blood supply, and (2) changes due to sepsis. Grossly, the hearts undergoing acute rejection appeared dusky red, edematous, and focally hemorrhagic. The histological appearance of acute rejection within the cardiac homografts (exclusive of conduction system morphology) was divided into five grades of increasing severity as follows:

Grade 0—No histological evidence of graft inflammation, degeneration, or recent necrosis.
Table 2
Summary of Findings in Three Dogs with Histological Evidence of Chronic but Not of Acute Rejection

<table>
<thead>
<tr>
<th>Dog</th>
<th>Survival time (days)</th>
<th>Treatment</th>
<th>Cause of death</th>
<th>Rejection grade</th>
<th>C.S. lesions</th>
<th>ECG</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA13</td>
<td>54</td>
<td>Azathioprine</td>
<td>Pneumonia</td>
<td>Mild chronic</td>
<td>None</td>
<td>Progressive decrease</td>
</tr>
<tr>
<td>LA6</td>
<td>61</td>
<td>Azathioprine</td>
<td>Pneumonia</td>
<td>Moderate chronic</td>
<td>None</td>
<td>Atrial flutter*</td>
</tr>
<tr>
<td>LA15</td>
<td>84</td>
<td>Azathioprine</td>
<td>Sepsis</td>
<td>Mild chronic</td>
<td>Focal fibrosis of A-V bundle and left bundle branch</td>
<td>Sinus bradycardia* Progressive decrease</td>
</tr>
</tbody>
</table>

* Arrhythmias reversed with therapy prior to death.

Table 3
Summary of Findings in Five Dogs with Cardiac Homografts Displaying No Evidence of Acute or Chronic Rejection

<table>
<thead>
<tr>
<th>Dog</th>
<th>Survival time (days)</th>
<th>Treatment</th>
<th>Cause of death</th>
<th>Conduction system lesions</th>
<th>ECG</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA12</td>
<td>3</td>
<td>ALS Azathioprine</td>
<td>Pneumonia</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>LA1</td>
<td>4</td>
<td>ALS</td>
<td>Myocardial infarct</td>
<td>Grade A</td>
<td>None</td>
</tr>
<tr>
<td>LA3</td>
<td>5</td>
<td>ALS Azathioprine Prednisolone</td>
<td>Hemolytic anemia</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>LA14</td>
<td>7</td>
<td>ALS Prednisolone</td>
<td>Myocardial infarct</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>LA13</td>
<td>18</td>
<td>ALS Azathioprine Prednisolone</td>
<td>Unknown</td>
<td>Endothelial proliferation of A-V nodal artery</td>
<td>First degree block* Decreased*</td>
</tr>
</tbody>
</table>

* Reversed with therapy prior to death.

Hearts with only scanty lymphocytic infiltrate were also considered grade 0.

Grade I—Mild to moderate perivascular mononuclear cellular infiltrate as the only consistent finding. Swelling of the capillary endothelial cells and focal capillary rupture were occasionally present. Vascular, valvular, or myocardial necrosis was absent.

Grade II—Endothelial cell swelling, edema, more widespread capillary rupture than in grade I, and occasional capillary and venous thrombi. Focal myocardial degenerative changes and valvular mononuclear infiltrates were often found. In addition these hearts contained a moderate to heavy perivascular infiltrate of mononuclear cells and a mild to moderate diffuse infiltrate of the same cell types.

Grade III—Widespread capillary rupture with scattered small foci of ischemic necrosis, segmental arteritis with endothelial swelling and transmural necrosis, and widespread degenerative lesions manifested by hydropic swelling of myocytes. These changes were accompanied by a heavy infiltrate of mononuclear cells and occasional polymorphonuclear cells.

Grade IV—Necrotic vascular and myocardial changes predominated with or without a heavy mononuclear infiltrate. A polymorphonuclear infiltrate was usually prominent, particularly if there were large multifocal areas of infarction.

Focal calcification, a nonspecific lesion seen in all grades of rejection, as well as in the autografts, was seen more frequently as the rejection grade increased. Myocytolysis, defined as loss of
myocyte substance with retention of the reticulin network, was found almost exclusively in hearts which had been successfully treated for rejection crises. Theoretically, it could have been found in all grades of acute rejection but in this series was found only in dogs with grade III or IV lesions.

Chronic rejection was diagnosed in the presence of arteritis combined with healed arterial lesions, intimal fibrosis with luminal narrowing, or both.

**Histological Assessment of the Conduction System**

In homografts undergoing rejection the lesions of the conduction system usually paralleled those in the myocardium; they were graded separately, however, because of their potential clinical significance. In all cases the A-V node, approaches to this node, the main A-V bundle, and the bundle branches were examined. Evaluation of the sinus node was a problem because of the node's proximity to the atrial suture line. The node was usually encompassed within the inflammatory response to the atrial anastomosis, and separation of inflammation due to surgical trauma from that due to rejection was not possible. Grading of conduction system lesions, therefore, was based on examination of the A-V node, the main bundle, and bundle branches. Histological grading of conduction system lesions was as follows:

- Grade 0—No demonstrable pathological changes.
- Grade A—Scant, perivascular mononuclear cell infiltrate in the A-V node, main bundle, or bundle branches. Necrosis of the sinus node was inconstantly present depending on proximity to the atrial suture line.
- Grade B—Moderate mononuclear and occasional polymorphonuclear cell infiltration of the A-V node, main bundle, or the bundle branches associated with edema. In addition to the infiltrate, focal hemorrhage was often present in the right or left bundle. Vascular lesions consisted of no more than endothelial cell swelling and mononuclear cell margination.
- Grade C—The A-V node and main bundle contained moderate to heavy mononuclear cellular infiltrate, frequent necrosis of the small vessels, but no necrosis of Purkinje fibers. Conduction systems with extensive infiltration and necrosis in either the right or left bundle branches were placed in this grade.

**Figure 1**

Left. A-V node (within arrows) in dog (LA17) dying at 26 days with acute rejection. H&E; x 50. Right. Higher magnification of A-V node shows massive lymphocytic infiltrate with separation and loss of A-V nodal fibers. H&E; x 320.
Grade D—No lesion of the conduction system was placed in this grade unless there was necrosis of the A-V node or main bundle.

Chronic lesions consisted of intimal proliferation or fibrosis in small vessels supplying the conduction system. Increased fibrosis of the A-V node, main bundle, or bundle branches in the presence of patent coronary arteries, and over that found in normal controls, was considered a chronic lesion.

Results

Correlation of Arrhythmias with Conduction System Lesions and Condition of the Graft

Postoperative survival ranged from 3 to 84 days. Although care was taken to avoid trauma to the sinus node and conducting tissue in the atrial septum, postmortem examination frequently revealed necrotic changes in the sinus node due to proximity to the atrial suture line. Nevertheless, all dogs exhibited an atrial rhythm resembling a normal sinus mechanism by the third postoperative day.

Acute Rejection of Homografts

A histological diagnosis of acute rejection was made in 12 of the 20 homografted animals. Lesions of the conduction system of grade B to grade D severity were found in each of these 12. The electrocardiographic features of rejection in this group included decreasing voltage in seven and arrhythmias in five. These arrhythmias were first and second degree heart block, intraventricular block, sinus arrest, atrial flutter, and nodal rhythm (table 1).

Grade D conduction system lesions were found in four dogs. These were associated with arrhythmias in all four and with decreasing voltage in three of the four. In two of these (LA7 and LA17), necrosis found within the A-V node was associated with massive proliferative mononuclear cell infiltrate (fig. 1). Polymorphonuclear cells were also found but were predominantly in contact with the endothelium and walls of the small nodal vessels.

Figure 2


Circulation, Volume XXXIX, May 1969
In the other two dogs with grade D lesions (LA8 and LA10) small necrotic foci of Purkinje fibers associated with a polymorphonuclear infiltrate were found in the conduction bundles but not in the A-V node (fig. 2). Both of these animals had heavy mononuclear cellular infiltration of the A-V node, endothelial cell swelling, medial hyalinization, and polymorphonuclear infiltration of the conduction system vasculature, and bilateral bundle-branch edema.

Grade C conduction system lesions found in three of the 12 animals were associated with arrhythmias in one instance and decreasing voltage in two. The one dog with arrhythmia (LA18) had a heavy mononuclear cell infiltrate in the A-V node and main bundle and vasculitis of the small nodal vessels but no necrosis (fig. 3).

Grade B lesions were found in the remaining five dogs; none showed arrhythmias, but two had decreasing voltage.

**Chronic Rejection of the Homografts**

Three of the 20 homografts had histological features of chronic rejection only. All three had shown significant decreases in electrocardiographic voltage, and two had developed multiple arrhythmias including sinus bradycardia, atrial flutter, and nodal rhythm reversed with therapy (table 2). Only the last-mentioned dog (LA15) had a lesion of the conduction system; namely, fibrosis of the conduction bundles.

**No Rejection of Homografts**

Four of the five dogs without histological evidence of rejection had unremarkable electrocardiograms after the second to third postoperative day. One, however, had shown diminished voltage, first degree heart block, and atrial bigeminy all reversed with steroid therapy before death. Atrial flutter which had supervened during this clinical rejection crisis remained fixed. The cause of death in this

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**Figure 3**

animal was not determined. There was no evidence of acute or chronic rejection, but there was intimal proliferation within the A-V nodal artery.

**Autografts**

None of the five autografts or three normal hearts showed myocardial or conduction system lesions resembling those of rejection. In all of these animals serial electrocardiograms were unremarkable.

**Histopathological Findings in Homograft Rejection**

In homografts undergoing rejection the most frequent conduction system finding consisted of a diffuse infiltrate of the A-V node and main bundle by mononuclear cells. These cells had small to large dense nuclei and abundant basophilic cytoplasm which displayed varying degrees of pyroninophilia. Mitotic figures and multinucleated forms were common. This round-cell infiltrate, including macrophages, often surrounded the nerve bundles and branches found within the atria and adjacent to the A-V node and main bundle. Perineural infiltrates were scanty in the autografts, mild in the homografts that were not undergoing rejection and invariably heavy in the acutely rejected homografts. In acute rejection the atrial septum was usually infiltrated to the same degree as the conduction system. Although all 12 homografts undergoing rejection showed myocardial infiltration, in only one was the infiltration of the myocardium adjacent to the main bundle. The connective tissue surrounding the main bundle and encompassing the right and left bundles was generally unremarkable. Endothelial cell swelling in vessels supplying the A-V node and conduction system was common in hearts undergoing acute rejection but was not seen in autografts or homografts without rejection. Hemorrhage and small vessel necrosis, consisting of medial hyalinization and transmural infiltration of polymorphonuclear and mononuclear cells, was frequently seen within the A-V node and main bundle in grade C and D lesions. Necrosis of the A-V node, present in two animals, was associated in both instances with a massive large mononuclear cell infiltrate. Focal necrosis of the A-V bundle was present in two instances.

**Discussion**

The relationship of pathological changes in the conduction system to arrhythmias and conduction disturbances is evident from established clinicopathological correlations in other disease states. In our present series of dogs the invariable involvement of the conduction system in acute rejection often led to arrhythmias which were recognized long before the development of heart failure. Indeed, recognition of the clinical consequences of immunological conduction system injury following cardiac transplantation in man has contributed significantly to the diagnosis and management of rejection and will be described fully in other reports.

It is somewhat surprising that the severity of histological lesions seen within our 12 acutely rejected canine cardiac homografts should not be associated uniformly with conduction disturbances or arrhythmias. The absence of consistent electrocardiographic abnormalities suggests evidence of a significant degree of functional reserve in conduction tissue. However, it should be emphasized that electrocardiographic monitoring in these dogs was relatively infrequent and obviously inadequate to rule out transient abnormalities. That such might have occurred is suggested by the acute rejection deaths in the early postoperative period of four animals whose electrocardiograms 12 to 24 hours earlier had revealed no diagnostic changes.

Another significant consideration is the possible reversal of functionally important pathological changes in the conduction system with appropriate therapy. This is illustrated by dog LA15 whose course included multiple arrhythmias and alterations in conduction during a well-established episode of threatened rejection. Despite the positive correlation of these electrocardiographic disturbances with conduction system pathology in other animals dying with rejection, the conduction system of this animal was free of acute pathology.
immunological damage following death due to sepsis. The etiology of arrhythmias in this dog is unknown; however, a likely cause is conduction system ischemia due to widespread endothelial swelling. On this basis the reversal of arrhythmias may be attributed to the anti-inflammatory effects of steroid therapy.

The immunopathological mechanisms responsible for the prominence of immune lesions within conducting tissue are unclear at the present time. However, the comparatively rich vascularity of the sinus node, atrioventricular node, and main bundle demonstrated by Clarke suggests at least partial explanation in view of the endothelial edema and predominantly perivascular distribution of mononuclear cells seen in early rejection.

It may be concluded that the histological findings within the conduction systems of rejected hearts represent a primary immunological response. No animal lacking general histological evidence of graft rejection showed infiltration of the conduction system. Diffusion of infiltrating cells from adjacent myocardium is eliminated as a primary mechanism by the consistent finding of significantly heavier infiltration within conduction tissue compared to the adjacent ventricular septum (fig. 4). In contrast, the diffuse mononuclear cell infiltrate contained within atrial septa was comparable in every instance to that within the conduction system. Finally, the negative findings in autograft hearts rule out a causal role for ischemic postsurgical changes or simple reaction to degenerating nerve elements.

References


Figure 4


Advance Through Experimental Medicine

When, in 1927, we arrived in the department of Carl Wiggers, one of our best teachers, he asked us first of all which experiments we had performed recently. Answering this question, we explained carefully our observations concerning the baroreceptors regulating arterial pressure and the chemoreceptors acting on respiration. Carl Wiggers listened and then said: "Heymans, do you really believe what you said? Because I suppose you know that is in full contradiction with classic opinions. Now, let us not argue, but tomorrow we shall provide a dog and you are going to demonstrate what you said." Just as John Hunter said to Edward Jenner: "Why think? Why not try the experiment?" Next morning, then, we performed the demonstration while Carl Wiggers was looking at it very carefully. At the end of the experiment, he said "Heymans, the dog is right, textbooks are wrong!"—Corneille J. F. Heymans: In Reflections on Biologic Research, edited by Giulio Gabbiani. St. Louis, Warren H. Green, Inc., 1967, p. 83.
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