Cardiac Fibrinoid Lesions Produced by Cross Circulation or Temporary A-V Shunts

By Richard L. Davis, M.D., Joel G. Brunson, M.D. and Peter E. Fehr, B.A.

Localized acute cardiac lesions containing fibrinoid were produced in rabbits by carotid-jugular cross circulation or temporary arteriovenous shunts, using small amounts of heparin as anticoagulant. Excessive amounts of heparin markedly reduced the incidence of fibrinoid in the lesions, which suggests that the formation of fibrinoid was inhibited or its deposition in the heart prevented. The results of the experiments suggest that the shunting procedures not only produce mechanical damage to the heart, but also participate in the production of fibrinoid or its precursors, which is deposited in localized sites of cardiac damage.

The occurrence of cardiac lesions in rabbits given intravenous gram-negative bacterial endotoxin, alone or in conjunction with bacterial infection or the administration of certain high molecular weight acidic polymers such as Liquoid, has been described in previous papers. These lesions consisted of myocardial and valvular hemorrhages, muscle necrosis, cellular reaction and the presence of hyaline fibrinoid material within the walls of the coronary arteries and the cardiac valves. Several recent papers have dealt with the nature and origin of this fibrinoid material.

In a previous study it was shown that typical fibrinoid material was deposited in the kidneys of rabbits perfused with the blood of properly prepared donor animals, indicating that this material or its precursors is carried by the circulating blood. A control group of rabbits in these studies, subjected to carotid-jugular cross transfusion, developed isolated cardiac fibrinoid lesions.

In rabbits given intravenous endotoxin, or endotoxin in association with Liquoid or bacterial infection, widespread extra-cardiac lesions also occur. The diffuse nature of these lesions, the rapidity of their development and the ischemic necrosis which occurs in many organs and tissues, is associated with a high mortality rate which precludes a long term study of the lesions. The observation that cardiac lesions were present in the control group of rabbits in the previously reported cross transfusion experiments suggested a method whereby such lesions might be produced and confined to the heart and studied over a longer period of time.

The present paper, therefore, deals with the production of cardiac lesions in rabbits by means of carotid-jugular cross circulation and, by a modification of that procedure, a temporary carotid-jugular arteriovenous shunt.

Materials and Methods

Seventy-nine hybrid albino rabbits of both sexes weighing 1 to 1.5 Kg. were used. They were fed Purina rabbit pellets and had free access to water. Details concerning the number of animals used are given in the text. The animals died or were killed within 48 hours after the shunting procedure. Routine postmortem examinations were performed and the tissues were fixed in 10 per cent neutral formalin. Sections were taken from the heart, lungs, kidneys, spleen, liver, adrenal glands and skeletal muscle. The hearts were sectioned after fixation, as previously indicated, to obtain multiple sections of the valves. Hematoxylin and eosin were used routinely, but many additional sections were stained by the periodic acid-Schiff method, Mallory's phosphotungstic acid hematoxylin and toluidine blue.

Heparin sodium (Parke-Davis) was used as the anticoagulant in the shunting procedures. It was injected in a concentration of 10 mg./ml. into the marginal ear vein of the animals just prior to cannulation of the neck vessels.

For cross circulation, the carotid artery of 1 animal was connected by means of polyethylene tubing (IntraMedic-Clay-Adams, PE 160, ID = 0.045", OD = 0.062", and PE 205, ID = 0.062"
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OD = 0.082) to the external jugular vein of a second animal, and the carotid artery of the second animal was connected to the jugular vein of the first in a similar manner. Details concerning this procedure have been given in a previous report. Cross circulation was allowed to continue for varying periods of time, but did not exceed 35 min. in any 1 pair. For arteriovenous shunt experiments, the carotid artery and external jugular vein of the same animal were connected by means of polyethylene tubing (same type and size as above), and circulation allowed to continue for a maximum of 30 min. Upon termination of the procedure, the tubing was checked for patency and free flow, the cannulae were removed and the neck vessels which had been cannulated were ligated.

Thirty animals were used as controls. Ten of these were given .10 mg. of heparin and the carotid artery and jugular vein cannulated, but shunting was not performed. Ten others were given 10 mg. of heparin and 10 were used as absolute controls.

RESULTS

The results (table 1) show that cardiac lesions occurred in a high percentage of animals subjected to the cross circulation or temporary arteriovenous shunt procedures. Although there was a marked decrease in the incidence of cardiac fibrinoid in the animals given 4.0 mg. of heparin in conjunction with cross circulation, the incidence of cardiac lesions and the mortality rate were not appreciably altered, indicating that the heparin either failed to prevent the lethal effects inherent in the procedure, or that such large amounts of heparin may have been a factor in producing death.

The cardiac lesions consisted of areas of muscle necrosis and calcification, mononuclear or heterophilic cellular reaction, and hemorrhages. Extensive areas of ischemic necrosis were observed in many animals, associated with a marked heterophilic cellular reaction. In other animals discrete perivascular areas of necrosis and mononuclear cellular reaction were present. Many of the cells in association with these lesions resembled Anitschkow cells and occasional multinucleate giant cells were observed.

Valvular hemorrhage was noted frequently, and it occasionally extended throughout the substance of the valve (figs. 1–3). In association with the hemorrhage, a cellular reaction composed of large mononuclear cells with indistinct borders and deeply basophilic cytoplasm and many Anitschkow cells, was commonly observed (fig. 4). These changes were almost invariably confined to the mitral and aortic valves.

Fibrinoid material was observed in the walls of the coronary arteries, in the valves, the pericardium, and the endocardium. This material was similar in location and in tinctorial characteristics to that described in previous papers. In the valves it was present within, or in close proximity to, areas of hemorrhage and cellular reaction. The cells were often palisaded around the fibrinoid material (figs. 1–3). In the coronary arteries, it usually appeared as a thin band of eosinophilic material lying beneath the endothelium of a segment of the vessel, although it sometimes was present throughout the entire thickness of the vessel and replaced large portions of the wall (figs. 5, 6). Occasionally fibrinoid was present in the perivascular spaces and less frequently it was observed as a thin layer of material along the pericardial surface, or projecting from the endocardium into the ventricular cavity.

Previous studies have shown that experimental fibrinoid lesions in rabbits are prevented by large amounts of heparin. In the present experiments cardiac fibrinoid was observed in only 1 animal in the group of 16 subjected to cross circulation using 40 mg. of heparin as anticoagulant. The incidence of nonfibrinoid lesions was not significantly changed, however (table 1).

In 70 per cent of the hearts of the control

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Duration of shunt* min.</th>
<th>Heparin mg.</th>
<th>No. of animals</th>
<th>Per cent dying within 12 hours</th>
<th>Cardiac lesions</th>
<th>Cardiac fibrinoid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross circ.</td>
<td>22</td>
<td>5, 10, or 20</td>
<td>22</td>
<td>32%</td>
<td>82%</td>
<td>45%</td>
</tr>
<tr>
<td>A-V shunt</td>
<td>24</td>
<td>40</td>
<td>16</td>
<td>37%</td>
<td>75%</td>
<td>6%</td>
</tr>
<tr>
<td>Carotid-jugular cannulation</td>
<td>30</td>
<td>10</td>
<td>11</td>
<td>18%</td>
<td>100%</td>
<td>27%</td>
</tr>
<tr>
<td></td>
<td>No shunt</td>
<td>10</td>
<td>10</td>
<td>0%</td>
<td>70%</td>
<td>0%</td>
</tr>
</tbody>
</table>

* Average duration of shunt for group.
Fig. 1. Section of mitral valve from rabbit subjected to cross circulation showing extensive hemorrhage throughout the substance of the valve together with fibrinoid deposition. X 250.

Fig. 2. Mitral valve from a rabbit following cross circulation. Extensive hemorrhage, edema, and a cellular reaction are present in association with a large mass of fibrinoid material within the substance of the valve. X 100.

Fig. 3. Higher magnification of a portion of valve in figure 2 showing the homogeneous nature of the fibrinoid material which lies in close proximity to an area of hemorrhage. Considerable nuclear debris is present. X 250.

Fig. 4. Mitral valve from a rabbit subjected to arteriovenous shunt. The valve is edematous and there is an inflammatory reaction consisting of large cells with indistinct borders and dark-staining fibrocytoid nuclei. X 250.
animals in which the carotid artery and jugular vein were cannulated, but shunting not performed, areas of muscle necrosis, cellular reaction and myocardial and valvular hemorrhages were observed, but no fibrinoid was noted. In the remaining controls, an occasional heart showed small areas of focal cellular reaction consisting of small mononuclear cells, similar to the lesions of spontaneous myocarditis.

In 1 of the animals subjected to cross circulation, microscopic examination of the lungs showed the presence of deeply acidophilic thrombi or emboli within branches of the pulmonary arteries. These thrombi or emboli were similar in tinctorial properties to the fibrinoid material present in the hearts. In none of the other animals, however, were any extra-cardiac lesions observed.

**Discussion**

The results of the present experiments indicate that isolated cardiac lesions can be produced in a high percentage of rabbits subjected to carotid-jugular cross circulation or temporary arteriovenous shunt procedures. These lesions consisted of muscle necrosis, mononuclear and heterophilic cellular reaction, and myocardial and valvular hemorrhages. In association with these changes fibrinoid material was observed in the walls of the coronary arteries, in the valves and perivascular spaces and in the endocardium and pericardium. In only 1 of 33 animals subjected to these procedures was any lesion seen in any other organ; this was in the lungs. It appears, therefore, that this technic does produce localized, fibrinoid-containing cardiac lesions.
The factors inherent in these technics which cause the production and deposition of this material are not known at the present time. In the control group of animals in which every procedure was performed except the actual shunting, 70 per cent showed cardiac changes, but in none of these was fibrinoid observed. It is probable that both mechanical and chemical factors play a role in the development of the lesions.

In view of previous reports concerning the prevention of experimental fibrinoid lesions by the use of excessive amounts of heparin, a group of rabbits was subjected to cross circulation using large amounts of heparin as anticoagulant. The results indicate that the amount of heparin used materially reduced the incidence of fibrinoid in the hearts but, as might be expected, did not significantly alter the incidence of nonfibrinoid cardiac lesions. This observation suggests that the excessive amounts of heparin either inhibited the production of fibrinoid or its precursors, or prevented the deposition of this material in the hearts. The results of the present experiments suggest, however, that cross circulation or temporary arteriovenous shunt technics not only provide mechanical damage to a single organ but also participate in the production of this material which is then deposited in localized sites of damage in the heart.

The morphologic similarity of these cardiac lesions to those produced by the intravenous administration of endotoxin or endotoxin in association with Liquoid and the decreased incidence of these lesions when the shunting procedures were performed with large amounts of heparin, suggest that cross circulation or temporary arteriovenous shunt technics not only provide mechanical damage to a single organ but also participate in the production of fibrinoid or its precursors, which is deposited in localized sites of cardiac damage. It is suggested that the excessive amounts of heparin may have inhibited the formation of fibrinoid, or prevented its deposition in the heart.

The production of such acute, localized cardiac fibrinoid lesions by means of these shunting procedures is of interest in view of the reported development of bacterial endocarditis in dogs in which permanent arteriovenous fistulas had been created. Preliminary unpublished studies indicate a tendency to development of chronic cardiac lesions in rabbits subjected to cross circulation, and also a marked increased susceptibility to experimental bacterial infection in the form of bacterial endocarditis following these procedures. These observations suggest that fibrinoid lesions of the coronary arteries and heart valves may precede the localization of bacteria in these areas, providing a continued "stimulus" for the development of chronic lesions.

**SUMMARY**

Localized acute cardiac arterial and valvular lesions containing fibrinoid were produced in rabbits by carotid-jugular cross circulation or temporary arteriovenous shunt procedures, using small amounts of heparin as anticoagulant. Excessive amounts of heparin markedly reduced the incidence of fibrinoid in the lesions, but did not significantly alter the incidence of nonfibrinoid cardiac lesions. The results suggest that the shunting procedures not only produce mechanical damage to the heart, but also participate in the production of fibrinoid or its precursors, which is deposited in localized sites of cardiac damage. It is suggested that the excessive amounts of heparin may have inhibited the formation of fibrinoid, or prevented its deposition in the heart.

**Summario in Interlingua**

Acute e localisate lesiones arterial e valvular del corde que contineva fibrinoide esseva producite in conilios per medio de circulation cruciate carotido-jugular o temporari derivatones arterio-venose con le uso de parve quantitates de heparina como anticoagulante. Excessive quantitates de heparina reduceva marcatemente le incidentia de fibrinoide in le lesiones sed non alterava significativamente le
incidentia de non-fibrinoide lesiones cardiac. Le resultatos pare indicar que le derivationes produce non solmente lesiones mechanic del corde sed participa etiam in le production de fibrinoide o su precursores, deponite in localisate sitos de lesion cardiac. Nos opina que le excessive quantitates de heparina serviva possibilemente a inhibir le formation de fibrinoide o preveniva su deposition in le corde.

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