ABSTRACT

Coronary blood flow and vascular reactivity were studied at intervals after donor dog hearts were transplanted into the necks of recipient dogs. Coronary blood flow was measured with an electromagnetic flow transducer on the carotid artery of the recipient dog, and coronary vascular reactivity was assessed from the response of the coronary vessels to the vasodilator drugs, dipyridamole and adenosine. During the first 4 days after the transplant, resting coronary blood flow and vascular resistance did not change. Responses to dipyridamole and adenosine were unchanged during the first 2 days but were reduced on the third and fourth days. Increased vascular permeability to circulating Evans blue dye and deposition of colloidal carbon in venules occurred in association with small increases in left ventricular myocardial water content 3 and 4 days after the transplant. These changes indicate that an inflammatory response accompanies the onset of histological evidence of rejection at 3 and 4 days. The reduced vascular reactivity of the transplanted heart at 3 and 4 days may be related to morphological changes in arterioles rather than to gross edema formation during rejection.

KEY WORDS coronary blood flow Evans blue dye coronary vascular reactivity permeability studies in the heart dipyridamole acute rejection adenosine colloidal carbon transplantation

The early phase of acute rejection of organ allografts is accompanied by evidence of increased vascular permeability (1, 2) and mononuclear cell infiltration. Similar changes are present in the early rejection of the transplanted heart (3-5), indicating alterations in the walls of the coronary blood vessels. The response of these altered vessels to coronary vasodilator drugs is not known but may be of clinical importance in the management of rejection crises associated with a reduced coronary blood flow (6).

In earlier studies we have shown that the response of the coronary vasculature to vasodilator drugs is normal in the immediate posttransplantation period (7, 8). In the present study, we measured the coronary blood flow in the heart from the time of the transplant until there was early histological evidence of rejection and we assessed coronary vascular reactivity by recording the increases in coronary blood flow produced by the vasodilator drugs, dipyridamole and adenosine. Changes in coronary artery reactivity were correlated with histopathological changes and permeability studies in coronary arterial and cardiac microcirculation in the early phases of rejection.

Methods

EXPERIMENTAL MODEL

The donor dog's heart was transplanted into the neck of the recipient dog according to a modification of the technique described previously (7). All the dogs were anesthetized with sodium thiopental (20 mg/kg, iv) and maintained on a gas mixture of nitrous oxide and oxygen (2:1). The recipient dogs (20-25 kg) also received halothane (0.5—1.0%).

The neck of the recipient dog was dissected to expose the carotid artery and the external jugular vein. Arterial and venous polyvinyl catheters (4 mm, o.d., and 3 mm, i.d.) were placed in the femoral artery and vein of the recipient dog and connected to large-bore (8.00 mm, o.d. and 5.00 mm, i.d.) Silastic rubber tubing. The chest of the donor dog was opened through a midsternal incision, and the left lung was removed. Loose ties were placed around the innominate and subclavian arteries, the aorta, the superior and inferior vena cavae, and the azygous vein. Following the administration of heparin (5000 units, iv), arterial and venous polyvinyl catheters (4 mm, o.d., and 3 mm, i.d.) were placed in the innominate artery and the superior vena cava of the donor heart and connected to the Silastic rubber tubing from the recipient dog. The donor heart was isolated by tying off the major vessels.
and the right lung and was then perfused through the recipient's femoral artery via the catheter attached to the donor innominate artery. Blood from the coronary circulation of the donor heart was returned to the recipient dog through the tubing between the donor superior vena cava and the recipient's femoral vein (9).

The donor heart (90–130 g) was then transplanted into the neck of the recipient dog by anastomosing the donor subclavian artery to the proximal end of the recipient's carotid artery and the donor left pulmonary artery to the recipient's external jugular vein. In the group of dogs studied during the immediate posttransplantation period (1–6 hours) the anastomosing vessels were not sutured but were connected by polyvinyl tubing (4 mm, o.d., and 3 mm, i.d., for the arterial inflow and 8 mm, o.d., and 6 mm, i.d., for the pulmonary outflow). The perfusion catheters from the femoral vessels to the donor heart were then removed. The donor heart continued to beat forcefully in sinus rhythm throughout the procedure. In this preparation, blood flows into the donor aorta from the recipient's carotid artery at a pressure equal to the recipient dog's systemic pressure. Since the superior and inferior venae cavae and the azygous veins of the donor heart are ligated, the coronary venous blood of the donor heart is returned to the recipient's circulation through the left pulmonary artery to the external jugular vein anastomosis.

Following transplantation the pericardium was removed and the neck incision was closed after a drainage tube (8 mm, i.d.) had been inserted. The dog was then allowed to recover. Daily injections of penicillin (1,000,000 units) and streptomycin (0.5 g) were given. Postoperative recovery was usually rapid and uneventful. The left ventricle, the right ventricle, and the left and right atria of the donor heart could be seen pulsating through the skin of the neck, and twice-daily assessment of the rate, rhythm, and force of contraction was made by palpation. No studies were performed on any transplants found to be dilated or beating poorly.

The coronary blood flow in this preparation was the net volume of blood per unit time that entered the coronary vessels from the aorta and, if the aortic valve was competent, was measured with an electromagnetic flow probe (IVM model FT2HS) placed on the supplying divided carotid artery (7) and connected to an electromagnetic flowmeter EMI (type 28). The perfusion pressure in the donor aorta was measured through a triple-lumen polyvinyl catheter (2.50 mm, o.d., and 0.96 mm, i.d.) inserted through the innominate artery, positioned above the aortic valve, and connected to a Statham pressure transducer (model P23BD). This catheter was also used for drug infusions. The donor electrocardiogram was recorded by direct myocardial electrodes attached to the left ventricle. The electrocardiogram and the pressure and flow signals were displayed on an oscilloscope screen and recorded on photographic paper with a multichannel recorder (Electronics for Medicine DR8). The coronary blood flow was measured as the area (by planimetry) under the flow signal per unit time and converted to milliliters per minute from the direct flow measurement and the calibration signal (7). The coronary vascular resistance was estimated as the mean donor aortic pressure divided by the coronary blood flow. The right atrium of the donor heart collapsed during the studies and the donor coronary sinus pressure and right atrial pressure were then assumed to be zero.

Different groups of dogs were studied immediately after transplantation (1–6 hours) and at 1, 2, 3, and 4 days after transplantation. At each study, we measured the coronary blood flow and assessed the responses of the donor heart to the coronary vasodilator drugs, dipyridamole and adenosine. The recipient dogs were sedated with droperidol (5–10 mg, i.m) and 5-mg increments were given at hourly intervals throughout the period of study (usually 2 hours). On reopening the neck incision prior to each study, localized edema of the surrounding skin and subcutaneous tissue was usually found with occasional small loculate effusions; the epicardium of the donor heart was tethered to the neck tissues by fibrin strands. There was no obvious infection, and the left ventricle and the left atrium of the donor hearts were not distended.

The base of the donor heart was then exposed, and the donor aortic catheter, the electrocardiogram electrodes, and the electromagnetic flow probe were placed in their respective sites. The drug studies were performed 30–60 minutes after initial sedation. Resting donor heart rate, perfusion pressure, and coronary blood flow did not change by more than ±10% during the 1–2 hours of study. To avoid the effects of any transient minor changes in coronary blood flow, each drug-induced alteration in coronary blood flow was compared with a 1-minute control flow immediately preceding the drug study. At the end of each study, the donor heart was removed, and the flow probe was calibrated on the artery in situ (7). Macroscopically the donor myocardium appeared normal. Thrombi were not found in either the donor aorta or left ventricle, and both anastomoses were patent. The recipient dog's heart was also removed for study.

HISTOPATHOLOGICAL STUDIES

Multiple blocks of tissues from right and left ventricles and right and left atria from both donor and recipient hearts were fixed in buffered 10% Formol saline. Paraffin sections were stained with hematoxylin and eosin and with acid fuchsin stain (10).

VASCULAR PERMEABILITY STUDIES

Vascular permeability of the transplanted heart was assessed by injecting a 2.5% (w/v) solution of Evans blue dye in 0.9% saline (1.0 ml/kg, iv) into the recipient dogs. Circulating albumin labeled with dye that exuded into the heart was estimated by the formamide extraction technique (11). One hour after the injection of dye, samples of donor and recipient hearts were selected, weighed, and incubated in known volumes of formamide at 37°C for 3 days. The optical density of the filtered formamide extract was measured spectrophotometrically at a wavelength of 620 nm, and the concentration of dye was assessed by reference to a standard graph on which the optical densities of known
concentrations of dye in formamide were plotted against concentration. The dye content (µg/g) of the recipient heart was subtracted from the value for the respective donor heart to allow for the quantity of residual dye within the circulatory tree of the test tissues.

The site of increased vascular permeability of the microcirculation of the grafted hearts was detected by injecting the recipient dog with colloidal carbon (1.0 ml/kg, iv) (Pelikan, Gunther Wagner Werke, Hanover, Germany, batch C11-1431A) (12). When free circulating carbon had been cleared by the reticuloendothelial system (usually 1 hour), specimens of recipient and donor hearts were fixed in buffered 10% Formal saline and cleared by a modification of the Spalteholz technique (13). Cleared specimens were examined for the deposition of colloidal carbon in the endothelium of abnormally permeable vessels with a stereodissecting microscope.

**ESTIMATION OF LEFT VENTRICULAR WATER CONTENT**

When pieces of a normal dog’s left ventricular myocardium were dried for 5 days at a constant temperature of 45°C, the calculated water content was 76.00 ±0.20% (mean±SE) of the original specimen weight. Further drying of the specimen for up to 10 days resulted in an additional weight loss of less than 0.5%. Therefore, the water content of all donor and recipient hearts was measured by weighing specimens dried for 5 days at a temperature of 45°C.

The donor heart was weighed and four pieces of fat-free myocardium were removed from the anterior wall of the left ventricle. Each piece of myocardium was weighed, minced finely with scissors, allowed to dry in a constant-temperature oven at 45°C for 5 days, and then reweighed. The mean weight loss of the four samples, expressed as a percent of the original weight, was the percent of tissue water in the original specimen (14, 15). In each preparation, four pieces of recipient left ventricle were treated in a similar manner and used as the control. These control values did not differ significantly from values obtained for normal, non-operated dogs’ hearts.

**DRUG STUDIES**

The drugs were injected into the aorta of the donor heart through the triple-lumen catheter placed above the aortic valve. The dipyridamole solution (50 µg/ml) was made up in 0.9% saline and was given as a constant infusion at the rate of 1 ml/min. A Braun constant-infusion pump and syringe was used for the infusion, and allowance was made for a catheter transit time of 30 seconds. Adenosine was given as a rapid injection.

Studies were performed on a total of 47 dogs: 8 dogs at 1 day after transplantation, 6 dogs at 2 days, 7 dogs at 3 days, and 6 dogs at 4 days. The results were compared with those obtained from 20 dogs in the immediate posttransplantation period.

For the analysis of the results, Student’s t-test was used to determine the significance of differences between grouped observations in each series of experiments.

**Results**

**DRUG STUDIES**

Resting values (means ± se) for the coronary blood flow, mean perfusion pressure, and heart rate of the donor hearts at the various times during the 4-day study period after transplantation are shown in Figure 1. The findings at 1-6 hours, which have been published previously (7), are included for comparison. Resting coronary blood flow was not different at 1-6 hours, 1 day, and 3 and 4 days; it was increased at 2 days, but heart rates were much faster at that time. Coronary blood flow/beat 100 g~ l remained relatively steady over the 4 days. The donor aortic mean perfusion pressure at 1-6 hours was higher than that at 1, 2, 3, and 4 days. Thereafter at 1, 2, 3, and 4 days the perfusion pressure remained unchanged (P > 0.10). Donor heart rates were likewise unchanged at 1-6 hours and at 3 and 4 days (P > 0.10); a significant increase appeared at 1 and 2 days.

The effects of 10-minute infusions of dipyridamole (50 µg/min) on coronary blood flow,
perfusion pressure, and heart rate at 1, 2, 3, and 4 days are also shown in Figure 1. The increases in coronary blood flow obtained with dipyridamole at 1 and 2 days were not significantly different from those obtained at 1-6 hours. However, at 3 and 4 days the responses to dipyridamole were significantly less than the responses at 1-6 hours ($P < 0.001$ for each). To establish that this difference was not due to the lower perfusion pressure at these times, the results at 3 and 4 days were compared with those at 1 day when the perfusion pressures were the same (Fig. 1). The responses to dipyridamole at 3 and 4 days were also significantly less than those at 1 day ($P < 0.01$ for each).

The increases in coronary blood flow obtained with rapid injections of 2.5, 5, and 10 $\mu$g of adenosine were studied at 1, 2, 3, and 4 days (Fig. 2). The increases in coronary blood flow obtained with the three concentrations of adenosine at 1 and 2 days were not significantly different from those obtained at 1-6 hours. However, the increases obtained at 3 and 4 days were significantly reduced from the immediate posttransplant results ($P < 0.001$) and from the 24-hour studies ($P < 0.01$).

To determine whether the reduced responses to dipyridamole and adenosine at 3 and 4 days represented the maximum coronary blood flow possible in the transplanted heart at these times, the following studies were performed. The peak increases in coronary blood flow produced by 10-minute infusions of dipyridamole (50 $\mu$g/min) and by injections of adenosine (10 $\mu$g) were measured in seven preparations from the 1-48-hour group (3 dogs at 1-6 hours, 1 dog at 24 hours, 3 dogs at 48 hours); these results were compared with those from a group of 6 dogs at 3 and 4 days (2 dogs at 3 days and 4 dogs at 4 days). Once the peak increase in coronary blood flow to each drug had been determined, the adenosine injection was repeated in the presence of the dipyridamole infusion. This procedure always resulted in a potentiated coronary blood flow response in the 1-48-hour group of $197 \pm 30\%$ (SE), which was a significantly greater peak increase in coronary blood flow than had been obtained with either drug alone ($P < 0.01$ or better). The potentiated peak increases in coronary blood flow at 3 and 4 days were however significantly smaller than those obtained in the 1-48-hour group ($131 \pm 21\%$) ($P < 0.05$). These responses were not compared with reactive hyperemic responses, and maximum coronary blood flow was not measured.

**HISTOPATHOLOGY**

Transplanted hearts removed at 6 hours and 1 and 2 days exhibited pericarditis characterized by a fibrinous exudate and an infiltration of inflammatory cells. The cellular infiltration was slight at 6 hours and consisted mainly of neutrophil polymorphonuclear leukocytes; however, at 2 days the mononuclear cell was dominant. These changes were consistent with a traumatic pericarditis evoked by grafting. Evidence of minor ischemic damage was present in some hearts 1 and 2 days after grafting; interstitial accumulations of polymorphonuclear leukocytes associated with swollen myocardial fibers that showed blurring of striations and patchy staining with acid fuchsin were observed. It was estimated that in the most severely damaged hearts less than 5% of the fibers had histopathological evidence of degeneration.

Unequivocal evidence of early rejection was seen in hearts 3 and 4 days after transplantation. The first mild signs were seen at 3 days as a patchy, focal infiltration of mononuclear cells in the perivascular sheaths of venules and veins (Fig. 3). At 4 days the mononuclear infiltration was denser and the foci were more extensive although still localized to the perivascular site; infiltration was also seen beneath the endocardium. The interstitial spaces surrounding the more heavily infiltrated...
zones were distended by edema. The atria exhibited the greatest density of cellular infiltration; the inflammation was milder but of equal intensity in the two ventricles. At 4 days after transplantation, arterioles were also abnormal. Figure 4 illustrates the most advanced abnormalities. A perivascular infiltration of mononuclear cells was associated with loss of definition of the muscle, which was also sparsely infiltrated with inflammatory cells. The endothelium appeared unusually prominent with plump endothelial cells projecting into the lumen, suggesting a hyperplastic response.

Unequivocal evidence of thrombosis was not seen, although abnormal quantities of fibrin and aggregates of platelets were observed on the endothelia of both arterial and venous radicles with increasing frequency at 4 days.

The recipient hearts were normal.

VASCULAR PERMEABILITY STUDIES

The presence of extractable Evans blue dye in the transplanted heart expressed in µg/g in excess of that extracted from the recipient heart indicates increased permeability of the coronary vasculature following transplantation. The results from six donor hearts tested after 6 hours to 4 days are summarized in Figure 5. No evidence of increased vascular permeability was detected in donor hearts at 6 and 60 hours. At 72 and 80 hours after transplantation, vascular permeability to Evans blue dye was markedly increased.

In dogs injected intravenously with colloidal carbon, labeling of venules in the superficial pericardium was seen 1 day after transplantation. This finding corresponded to the histological evidence of surgical pericarditis. Thereafter, vascular labeling was not seen until 72–96 hours. At this
time, heavy carbon labeling of venules and veins was seen in irregular distribution throughout the transplanted heart. The carbon was incorporated in and on the endothelium (Figs. 6 and 7). No evidence of arterial or arteriolar labeling was seen nor was there significant evidence of luminal plugging with carbon. There was no carbon labeling in the recipient hearts.

**LEFT VENTRICULAR WATER CONTENT**

The left ventricular water content of 25 recipient dog hearts from 1 hour to 4 days after transplantation was 76.74 ± 0.29% (SE). Donor hearts from the 1-24-hour and the 2-, 3-, and 4-day groups were studied and compared with the recipient hearts from the same preparations. As shown in Table 1 there were small but insignificant differences between the water content of the normal recipient hearts and that of the transplanted hearts at 1-24 hours and 2 days after transplantation. However, a significant increase in myocardial water content of 1.9% was obtained at 3 days (P < 0.025) and of 2.1% at 4 days (P < 0.001).

**Discussion**

In this experiment, we transplanted a beating heart that was perfused continuously and was not subjected to an anoxic period. The donor hearts continued to beat strongly at a regular rate, and there was no left ventricular distention. The donor aorta and the left and right ventricles were free of clots up to 4 days after transplantation. The coronary blood flow measured immediately after transplantation (84 ± 5 [SE] ml/min 100 g⁻¹) was in the range obtained by Gregg (16) in the intact anesthetized dog (65–100 ml/min 100 g⁻¹). We concluded that the preparation was suitable for the study of changes in coronary blood flow and coronary vascular reactivity at different times after transplantation (7). To measure these changes, we chose dipyridamole and adenosine because of their potent coronary vasodilator actions in both normal hearts (17-23) and in acutely transplanted dog hearts (7). Furthermore, adenosine may be an important regulator of coronary blood flow (24, 25) and its actions can be potentiated by dipyridamole (7, 17).

The major findings of the present investigation were that the onset of rejection was accompanied by a significant reduction in coronary vascular reactivity as judged by responses to the two powerful coronary vasodilator drugs used and that this response occurred without any change in resting coronary blood flow or coronary vascular resistance. Stinson and his associates (5) recently reported the results of serial studies of coronary blood flow and coronary vascular resistance during rejection of orthotopically transplanted dog hearts. They also found only minor changes in resting values during the first 3 days after transplantation. In the four dogs with unmodified rejection that they studied, coronary blood flow decreased and coronary vascular resistance increased only during the 24-hour period immediately before the death of each dog from acute rejection. They also found reduced reactive hyperemic responses to 10-second coronary artery occlusions at the same time as these
TABLE 1

Estimation of Left Ventricular Water Content following Transplantation

<table>
<thead>
<tr>
<th>Time of study (hrs)</th>
<th>N</th>
<th>Donor</th>
<th>Recipient</th>
<th>Change (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% Water content</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-24</td>
<td>8</td>
<td>77.05 ± 0.58</td>
<td>76.49 ± 0.65</td>
<td>0.54 ± 0.13</td>
<td>&gt; 0.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(74.32-78.40)</td>
<td>(73.50-78.40)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>5</td>
<td>76.80 ± 0.64</td>
<td>76.00 ± 0.50</td>
<td>0.79 ± 0.23</td>
<td>&gt; 0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(73.70-77.60)</td>
<td>(72.70-77.60)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>7</td>
<td>78.92 ± 0.62</td>
<td>78.96 ± 0.58</td>
<td>1.96 ± 0.62</td>
<td>&lt; 0.025</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(75.85-80.78)</td>
<td>(75.00-80.75)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>96</td>
<td>5</td>
<td>79.67 ± 0.28</td>
<td>77.60 ± 0.13</td>
<td>2.07 ± 0.28</td>
<td>&lt; 0.0005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(78.86-80.45)</td>
<td>(77.56-80.06)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All values are means ± SE. Parentheses indicate range.

changes developed; the reactive hyperemic responses were not reduced significantly before this time. The present study extends these findings and establishes that coronary vascular reactivity measured by dipyridamole and adenosine is markedly reduced before any changes in resting coronary blood flow and coronary vascular resistance occur.

The values for resting coronary blood flow in this study and in a previous study (7) were smaller than those reported by Lee and Webb (26) and by Reemtsma et al. (27). They measured the pulmonary artery outflow of the transplanted heart and recorded mean values of 128 and 160 ml/min 100 g⁻¹, respectively. The effect of hypoxia may have been responsible for these higher values, since the Downie (28) technique of transplantation was used in both of these studies and a period of cardiac anoxia is unavoidable with this method. Semb (29), however, recorded coronary sinus blood flows of 54 and 67 ml/min 100 g⁻¹ in two transplanted hearts following a modified Downie technique. The only other reported study in which transplantation was effected without subjecting the donor heart to a period of anoxia was that of Boake and Folts (30). They recorded pulmonary arterial outflow values of the same order as the coronary blood flow measured in the present study.

Although our finding that resting coronary blood flow and coronary vascular resistance remained unchanged during the first 4 days after transplantation is in accord with the observations of Stinson and his associates (5), it is at variance with the results of the few other studies in which these observations have been made (6, 29, 31). The coronary blood flow of the transplanted heart has been shown either to decrease progressively following transplantation (6) or to increase significantly 2 days (31) and 4 days after transplantation (29). The difference between other published observations on coronary blood flow and the observations in the present paper and in that of Stinson and his associates (5) cannot be explained.

We did not investigate directly the mechanisms involved in producing this altered reactivity, but certain inferences can be drawn from the histopathological changes and the study of heart water content. The endothelial changes in the arterioles and venules were prominent 3 and 4 days after transplantation but these changes were not accompanied by histological evidence of vascular thrombosis. Using a similar type of preparation, Rowlands and his associates (4) noted the onset of histological evidence of rejection at the same time after transplantation as in our study and described similar alterations in arterioles and venules without widespread occurrence of vascular occlusion. Similar responses for orthotopic cardiac transplants have been reported by Stinson and his associates (5). It seems unlikely therefore that the diminished vascular reactivity we found results primarily from multiple small vessel occlusions; however, the degenerative changes seen in the coronary arteriolar walls may well limit the capacity of these vessels to respond to stimulation when rejection is in progress.

Increased vascular permeability and edema formation constitute an early indication of the onset of rejection (1-3). Evidence of rejection was found in the measurements we made of heart water. The values we found in normal dogs and recipient dogs were of the same order as those reported by Moulder et al. (32) and Ziegler and Goresky (15). The water content of the donor heart was not significantly different from these control values for up to 48 hours after transplantation (Table 1), suggesting that denervation of the heart did not affect the myocardial water content. The increases in heart water measured in the donor hearts on days 3 and 4 were small, but they were highly significant.
CORONARY CIRCULATION AND HEART REJECTION

and coincided with the appearance of histological evidence of rejection. Rodbard (33) has suggested that rapid fluid exchanges between the capillaries and the perivascular spaces may be important for the local regulation of blood flow. The accumulation of edema fluid in the perivascular space increases extravascular pressure and reduces the caliber of vessels as Dempster (34) and Stinson et al. (5) have pointed out. The differences in heart water that we measured on days 3 and 4, although highly significant, were very small indeed: increases of 1.9% and 2.1%, respectively, in hearts weighing 90-130 g. Thus it seems unlikely that these small increases could have been responsible for the large reductions in vascular reactivity. It seems more likely that the explanation lies in the structural alterations which occurred in the vessel walls themselves.

The use of intravenous Evans blue dye as an indicator of inflammatory exudation is well established (35). The dye preferentially binds circulating plasma albumin and, in the normal circulatory tree, the escape of albumin labeled with dye through the endothelial barrier into the interstitial space is sluggish. During an inflammatory response, albumin exudes freely through the endothelium, and the magnitude of such exudation can be readily quantified by extracting the dye in formamide (11). In the present study, the dye content of the recipient's heart, representing intravascular dye and the small amount of physiological leakage into the interstitial space, was subtracted from the value for the donor heart. An excess of dye content in the donor, therefore, indicates either a significant intravascular pooling of blood in the microcirculation of the donor or a significant increase in extravascular albumin due to increased permeability of vessels in the donor heart. The former explanation appears to have been excluded by the coronary flow and histopathological studies, and the dye accumulation strongly points to a significant episode of inflammatory exudation. In the seven experiments reported, exudation was detected at 72, 80, and 96 hours, with maximum exudation at 80 hours; however, at 6 and 60 hours, less dye was present in the donor heart compared with that in the recipient heart (Fig. 5). The latter findings suggest that a slightly diminished microcirculatory perfusion or a decrease in intravascular space existed in the donor heart, possibly related to the smaller work load on the donor heart compared with that on the recipient heart. The extent of exudation was less at 96 hours than at 72 hours.

Although the number of observations are small, the time course of exudation evoked by the process of cardiac rejection may be quite short lived, similar to that reported for cutaneous allografts (2).

Majno et al. (12) have shown that circulating colloidal carbon is a reliable marker for vessels exhibiting increased permeability. Circulating carbon administered at the dose described is rapidly cleared (usually within 1 hour) from the circulation by the reticuloendothelial system. Carbon is incorporated beneath the endothelium of inflamed vessels marking the site of exudation of albumin through the vessel walls. Normal vessels remain unmarked. Carbon is seen only in thin-walled vessels that lack recognizable muscle and that correspond to microcirculatory entities described as venules and collecting venules (12). The extent of carbon labeling of the venous side of the transplant microcirculation at 72 and 96 hours (Figs. 6 and 7) and its absence in the recipient heart confirmed the dye studies, which indicated a significant phase of inflammatory exudation accompanying rejection. The carbon labeling also confirmed that a significant proportion of the increased water content of the donor heart was an exudate rather than a simple transudate. These findings further support our hypothesis that the diminished coronary vascular reactivity we found may be related to morphological changes in arterioles rather than to the accumulation of gross edema during rejection.

Acknowledgment

We wish to thank Dr. D. Brender and Dr. H. J. H. Colebatch for reviewing the manuscript. Our thanks are also due to Mr. Alan Needham for technical assistance and Mrs. Pat Wilde for secretarial assistance.

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Studies on the Coronary Circulation during Rejection of the Heterotopically Transplanted Dog Heart
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doi: 10.1161/01.RES.33.2.224

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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