Acute Effects of Cardiac Glycosides on Aldosterone Secretion in Dogs with Hyperaldosteronism Secondary to Chronic Right Heart Failure


With the surgical assistance of Alfred Casper

Since the report of Deming and Luetscher that patients with congestive heart failure excrete abnormally large amounts of a salt-retaining substance in the urine, considerable attention has been focused on the role of aldosterone in congestive heart failure. Although there is a large body of evidence to demonstrate that excessive aldosterone secretion plays an important role in the marked Na retention in congestive heart failure, some investigators have failed to find increased secretion of this mineralocorticoid in clinical heart failure. The mechanisms leading to excessive aldosterone production in cardiac failure and the mechanisms by which aldosterone secretion is decreased as cardiac compensation is achieved have been the subject of much recent investigation. Although cardiac glycosides have been reported to reduce adrenal blood flow and aldosterone secretion in normal dogs, the possibility must be strongly considered that improved cardiovascular function leads to the decrease in aldosterone production after digitalization in heart failure.

The present study was designed with a dual purpose: first, to make a definitive evaluation of the aldosterone secretion rates in animals with experimental congestive heart failure, and secondly, to study the effect of rapid digitalization on the aldosterone secretion rates of dogs in frank congestive heart failure.

Methods

Experimental right heart failure was produced as described below in eight mongrel dogs, weighing 15 to 20 Kg. In addition, studies were performed on one eight-year-old hunting dog with spontaneously occurring congestive heart failure. The eight dogs with experimental congestive heart failure were kept in metabolic cages and maintained on a synthetic diet containing 60 mEq. of Na and 18 mEq. of K per day for at least two weeks prior to the acute studies. The dog with spontaneously occurring heart failure received a diet containing approximately 80 mEq. of Na and 80 mEq. of K per day during the week preceding the acute studies. Urinary Na excretion was determined by flame photometry. Right atrial pressures were measured at frequent intervals with a water manometer attached to a polyethylene catheter which was inserted via the femoral vein into the right atrium. All pressures were referred to a level 6 cm. above the table surface. Aldosterone and corticosterone secretion rates were determined by a method described previously, using the double isotope derivative technique of Kliman and Peterson.

In six dogs, right heart failure was produced by progressive pulmonic stenosis by the method of Davis, Hyatt, and Howell. A nylon ligature was placed around the main pulmonary artery trunk, and then tightened in two or three stages at one-week intervals until signs of right-sided congestive heart failure ensued. Three to five days after the final tightening of the pulmonary artery ligature, the right adrenolumbar vein was cannulated under light Na pentobarbital anesthesia; no fixed dose of anesthesia was given because dogs with heart failure vary markedly in their response to anesthetics. Two groups of control samples of adrenal venous blood were then collected at 30- to 50-minute intervals. All blood removed throughout this study was immediately replaced by homologous transfusion. Following control collections, each of the dogs was given 1.25 to 1.50 mg. of digoxin intravenously. Samples of adrenal effluent were then collected at 30- to 60-minute intervals for the three-
TABLE 1

Average Values for the Effects of Uigoxin on Steroid Secretion and Cardiovascular Function in Dogs with Congestive Heart Failure Secondary to Controlled Progressive Pulmonic Stenosis*

<table>
<thead>
<tr>
<th></th>
<th>Aldosterone secretion (µg./min.)</th>
<th>Corticosterone secretion (µg./min.)</th>
<th>Adrenal blood flow (ml./min.)</th>
<th>Mean arterial pressure (mm. Hg)</th>
<th>Inferior vena cava pressure (mm. H2O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.094</td>
<td>2.25</td>
<td>2.14</td>
<td>110</td>
<td>169</td>
</tr>
<tr>
<td>1 hour after digoxin</td>
<td>0.058</td>
<td>2.80</td>
<td>1.92</td>
<td>122</td>
<td>111</td>
</tr>
<tr>
<td>90 to 180 minutes</td>
<td>0.038</td>
<td>2.29</td>
<td>1.70</td>
<td>126</td>
<td>102</td>
</tr>
<tr>
<td>after digoxin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 hour after</td>
<td>0.092</td>
<td>2.69</td>
<td>1.85</td>
<td>81</td>
<td>187</td>
</tr>
<tr>
<td>pulmonary artery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ligature tightened</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal dogs</td>
<td>0.024</td>
<td>2.24</td>
<td></td>
<td>±0.017</td>
<td>±0.84</td>
</tr>
<tr>
<td>stressed by</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>laparotomy (N=20)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*A more detailed form of this table has been deposited as document number 6848 with the ADI Auxiliary Publications Project, Photoduplication Service, Library of Congress, Washington 25, D. C. A copy may be secured by citing the document number and by remitting $1.25 for photoprints, or $1.25 for 35 mm. microfilm. Advance payment is required. Make checks or money orders payable to: Chief, Photoduplication Service, Library of Congress.

Averages include only dogs 1 through 4, since dog 5 expired shortly after further constriction of the pulmonary artery, and no postdigoxin collections were obtained in dog 6.

hour period following the digoxin injection. Following the three-hour collection, in five of the six dogs the pulmonary artery ligature was tightened until the mean right atrial pressure (RAP) exceeded 180 mm. of water. Samples of adrenal venous blood were then collected one hour after pulmonary artery reconstruction. During each acute experiment, the arterial pressure was measured with a Statham strain gauge attached to an indwelling abdominal aortic catheter and was recorded continuously on a Sanborn apparatus. Right atrial pressure was determined by an indwelling polyethylene catheter connected to a water manometer.

In two dogs, heart failure was produced by a modification17 of the technique described by Borger et al.17 Tricuspid insufficiency was produced surgically by cutting the chordae tendineae of the tricuspid valve. Two to seven months after this procedure, a nylon ligature was placed about the main pulmonary artery of each of these dogs. No subsequent tightening of the pulmonary artery ligature was required to produce right heart failure, characterized by sustained elevation of RAP and almost complete Na retention, in either of these animals. Three to five days after the pulmonary artery ligature had been placed, adrenal effluent was collected before and after digoxin administration, as described above.

One dog, an eight-year-old, 20-Kg. pointer, had collapsed while hunting and his disorder had been diagnosed by his owner, a physician, as being congestive heart failure. This animal was then maintained on digitoxin and a variety of diuretics over the following three months; despite therapy, the dog continued rapidly to accumulate ascitic fluid and required paracentesis at two-week intervals during this time. Digitoxin was discontinued one month prior to the present studies, and chlorothiazide was the only medication which the animal received during this period. Right atrial, right ventricular, and pulmonary wedge pressures were determined prior to adrenal vein cannulation via standard catheterization technique. The right adrenolumbar vein was then cannulated under Na pentobarbital anesthesia, and four control collections of adrenal venous blood were obtained over a 30-minute period. Following control collections, the animal was given a total of 1.0 mg. of ouabain intravenously over a 30-minute period. Two more samples of adrenal effluent were collected 90 to 120 minutes after the beginning of the ouabain injection.

Results

CONTROL HEMODYNAMIC STUDIES IN EXPERIMENTAL AND NATURALLY OCCURRING RIGHT HEART FAILURE

In the six animals in which controlled progressive pulmonic stenosis was produced, all dogs appeared in excellent health and none developed signs of right ventricular failure following the initial placement of the pulmo-
Dogs with Right Heart Failure Secondary to Combined Tricuspid Insufficiency and Pulmonic Stenosis

<table>
<thead>
<tr>
<th>Dog</th>
<th>Time (min.)</th>
<th>Aldosterone secretion (µg./min.)</th>
<th>Corticosterone secretion (µg./min.)</th>
<th>Adrenal blood flow (mL/min.)</th>
<th>Mean arterial pressure (mm. Hg)</th>
<th>Inferior vena cava pressure (mm. Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>0</td>
<td>0.153</td>
<td>3.72</td>
<td>1.80</td>
<td>75</td>
<td>190</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>0.189</td>
<td>4.22</td>
<td>2.33</td>
<td>82</td>
<td>188</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.226</td>
<td>4.22</td>
<td>1.52</td>
<td>75</td>
<td>190</td>
</tr>
</tbody>
</table>

Digoxin (1.35 mg., I.V.)

|     | 40          | 0.295                            | 5.60                                | 2.33                        | 82                            | 195                           |
|     | 70          | 0.292                            | 3.13                                | 0.90                        | 83                            | 192                           |
|     | 100         | 0.247                            | 3.67                                | 0.90                        | 87                            | 195                           |
|     | 145         | 0.140                            | 2.02                                | 1.96                        | 122                           | 208                           |
| 8   | 0           | 0.100                            | 1.61                                | 1.63                        | 133                           | 206                           |
|     | 24          | 0.100                            | 1.61                                | 1.68                        | 132                           | 209                           |
|     | 30          | 0.099                            | 1.72                                | 1.68                        | 132                           | 209                           |

Digoxin (1.25 mg., I.V.)

|     | 40          | 0.050                            | 1.24                                | 1.87                        | 136                           | 212                           |
|     | 110         | 0.074                            | 1.61                                | 2.01                        | 135                           | 218                           |
|     | 150         | 0.090                            | 1.43                                | 1.71                        | 136                           | 218                           |
|     | 156         | 0.100                            | 1.58                                | 1.82                        | 136                           | 218                           |
|     | 162         | 0.096                            | 1.42                                | 1.93                        | 136                           | 218                           |

In the dog with naturally occurring congestive heart failure, preoperative studies showed atrial fibrillation with a ventricular rate of 200 per minute, massive cardiomegaly (total heart volume by x-ray was 52 cc./Kg., or three times normal), and a mean frontal plane electrocardiographic axis of +90 degrees. Catheterization revealed a mean right atrial pressure of 182 mm. of water, a right ventricular pressure of 47/8 mm. Hg, a mean pulmonary artery pressure of 32 with extremes of 47/25 mm. Hg, and a mean pulmo-
nary wedge pressure of 14 mm. Hg. Subsequent pathological examination of this dog revealed generalized cardiomegaly, scarring of both the tricuspid and the mitral valves, and the presence of 24 heart worms (Dirofilaria immitis) in the right ventricle and the main trunk of the pulmonary artery. The high pulmonary wedge and pulmonary arterial pressures, together with the pathological finding of a scarred mitral valve, suggest that right ventricular failure occurred as a result of pulmonary hypertension secondary to mitral valve disease; the possibility must also be considered that Dirofilaria immitis infestation had resulted in pulmonary endarteritis with consequent pulmonary hypertension and cor pulmonale.10

**ALDOSTERONE SECRETION IN EXPERIMENTAL AND NATURALLY OCCURRING RIGHT HEART FAILURE**

In each of the six dogs with controlled progressive pulmonic stenosis, the control aldosterone secretion rate was clearly elevated above the average value for normal dogs (fig. 1, table 1). The average value for the group of six dogs with heart failure secondary to controlled pulmonic stenosis was 0.089 mg./min., which represents a 267 per cent increase over the average secretion rate of 0.024 mg./min. observed previously in normal dogs during use of identical experimental and chemical methods. This difference is highly significant statistically (t = 3.85; P < 0.01).

In the dogs with combined tricuspid insufficiency and pulmonic stenosis (table 2), the control aldosterone secretion rates were likewise markedly elevated. In dog 7, which had ascites for seven months prior to the steroid secretion studies, the average control aldosterone secretion rate of 0.186 mg./min. was twice as great as the mean control value for the remainder of the dogs in the present study; while in dog 8, which had been forming ascites for six weeks prior to the acute studies, the aldosterone secretion rate approximated that of the animals with right heart failure secondary to controlled pulmonic stenosis. In the dog with naturally occurring heart failure, the four control values for aldosterone output were also very high (fig. 2), and the mean value of 0.085 mg./min. was essentially the same as that observed in the group of dogs with right heart failure secondary to controlled pulmonic stenosis.

**HEMODYNAMIC EFFECTS OF DIGITALIZATION IN DOGS WITH RIGHT HEART FAILURE**

In one of the animals with controlled progressive pulmonic stenosis, ventricular fibrillation occurred shortly after the injection of 1.5 mg. of digoxin. In each of the remaining five dogs with pulmonic stenosis, a definite increase in arterial pressure and a marked decrease in right atrial pressure occurred following digoxin administration. The increase in arterial pressure and the decrease in RAP were observed within 10 minutes after digitalization. The mean arterial pressure increased sharply during the first 15 minutes after the glycoside injection, reached a maximum level (from 124 to 135 mm. of Hg) between 60 and 90 minutes after digoxin administration, and persisted at the higher level until further pulmonary artery constriction was effected (table 1, fig. 3). The RAP decreased progressively during the first hour after the digoxin injection reached a minimum level (from 96 to 110 mm. of water) between 60 and 90 minutes after digitalization.
FIGURE 2
Effects of ouabain injection on aldosterone and corticosterone secretion rates, adrenal blood flow, inferior vena cava pressure, and mean arterial pressure in a dog with naturally occurring right heart failure.

and remained at this level until further constriction of the pulmonary artery ligature was performed (table 1, fig. 3). Following subsequent tightening of the pulmonary artery ligature, an immediate fall in arterial pressure (to levels of 82 to 96 mm. of Hg) and a marked rise in venous pressure (to 175 to 206 mm. of water) occurred in all five dogs. In four of the five dogs (dogs 1 through 4 of table 1) the arterial and venous pressures stabilized at the new levels, with very little variation during the remaining 90 minutes of observation. In the fifth dog, the arterial pressure showed a progressive decline after the final tightening of the pulmonary artery ligature, and this animal expired before further collections of adrenal venous blood could be made.

During the two-hour period following administration of digoxin to the dogs with combined tricuspid insufficiency and pulmonic stenosis, slight increases in RAP were observed and minimal increases in mean arterial pressure could be detected on the pressure tracings in both animals (table 2).

In the dog with naturally occurring heart failure, administration of ouabain led to a rise in mean arterial pressure from an average control value of 88 mm. Hg to a maximum of 110 mm. Hg, and a marked fall in RAP from a control level of 140 mm. water to a minimum of 38 mm. water. An increase in arterial pressure and a decrease in RAP were observed within five minutes after the beginning of the ouabain injection; these changes were maximal 40 to 50 minutes after the onset of glycoside administration (fig. 2).
increase (dog 7) and a slight decrease (dog 8) in aldosterone output occurred one hour after digoxin injection, but the 100-minute postdigeroxin values were essentially the same as the control values in both animals (table 2). Corticosterone secretion rates remained at the high level characteristic of normal dogs stressed by laparotomy throughout both experiments. Adrenal blood flow remained essentially constant throughout all periods in dog 8, but showed a significant (50 per cent) decrease 60 to 100 minutes after digoxin injection in dog 7; it is noteworthy that no change in aldosterone output occurred concomitant with the marked diminution in adrenal blood flow in dog 7.

Ouabain administration to the dog with naturally occurring heart failure led to a marked fall in aldosterone secretion rate to 25 per cent of the control value at 55 minutes and a further decrease to 17 per cent of the control value at 75 minutes after the onset of the glycoside injection (fig. 2). Corticosterone secretion remained at the elevated control level throughout the experiment, despite a 50 per cent fall in adrenal blood flow following the glycoside administration (fig. 2).

Discussion

The present experimental data demonstrate that hypersecretion of aldosterone is a consistent finding in dogs with experimental congestive heart failure secondary to controlled progressive pulmonic stenosis or to combined tricuspid insufficiency and pulmonic stenosis, and is also present in naturally occurring congestive heart failure in the dog. It should be pointed out that in this series of experiments, Na retention was virtually complete in all animals with heart failure at the time the aldosterone secretion rates were determined. These results confirm the earlier studies, in which the aldosterone secretion by dogs with experimental right heart failure was measured by a biological assay technique. In the present study, the control aldosterone secretion rate in one of the dogs with combined tricuspid insufficiency and pulmonic stenosis was considerably higher than the secretion rates in the other seven dogs with experimental right heart failure. This finding probably reflects the fact that this animal had been forming ascites for a longer period of time (seven months compared with 3 to 42 days for the other seven dogs) prior to the time of the acute studies, and consequently had more marked hypertrophy of the adrenal zona glomerulosa with a resultant greater capacity for aldosterone production. The observation that aldosterone output may increase with the duration of the clinical course of ascites has previously been made in regard to dogs with thoracic inferior vena cava constriction.

The failure of Driscoll and associates to find increased aldosterone output in dogs with controlled progressive pulmonic stenosis apparently resulted from the absence of cardiac failure in their animals at the time that adrenal effluent was collected; this explanation is suggested by the fact that the venous pressures in their experimental animals ranged from 63 to 112 mm. of saline. In the present experiment, as in earlier studies, no animal with a right atrial pressure less than 140 mm. of water exhibited the virtually complete Na retention which is characteristic.
of frank congestive heart failure. As pointed out previously, a stable state of cardiovascular hemodynamics is rarely observed following the production of right ventricular failure by progressive pulmonic stenosis. It appears likely that, in all of the six dogs reported by Driscoll et al., cardiac compensation had occurred prior to the time of adrenolumbar vein cannulation; no measurements of Na excretion were reported by these investigators.

Muller and associates reported normal rates of urinary aldosterone excretion in five of six patients with congestive heart failure. However, four of these five patients exhibited normal Na balance at the time the aldosterone excretion studies were performed. Patients with congestive heart failure and edema may, at times, have normal urinary Na excretion; at some point in the course of congestive heart failure, Na balance may occur at a new steady state, during which the volume of extracellular fluid, although markedly increased, remains fairly constant for several days or weeks. Increased secretion of aldosterone would not be expected at a time when such a patient was in Na balance. A physiological basis for the new steady state, in which the patient with cardiac decompensation exhibits Na balance and a normal rate of aldosterone production, was suggested by Davis and Ball. These investigators found that application of a body cast to dogs with virtually complete Na retention secondary to thoracic caval constriction invariably resulted in decreased urinary aldosterone excretion and increased Na excretion; they then postulated that aldosterone secretion was decreased as the result of an increase in intra-abdominal pressure which inhibited filtration of fluid into the peritoneal cavity. It appears reasonable to extend this explanation to patients with congestive heart failure and ascites; thus, when edema fluid accumulates in cardiac patients to the extent that increased tissue pressure prevents further filtration of fluid, a fall in aldosterone secretion and an increase in Na excretion may ensue. This sequence of events might account for the finding of a normal aldosterone secretion rate in one out of two patients with congestive heart failure reported by Ulick, Laragh, and Lieberman. Available evidence suggests that aldosterone production is consistently increased during periods of Na retention in patients or animals with congestive heart failure.

In the present experimental dogs with right heart failure secondary to controlled progressive pulmonic stenosis, improvement in cardiovascular hemodynamics as manifest by a decrease in RAP was observed within 10 to 15 minutes after digoxin administration; the hemodynamic response was similar to that reported previously in the same animal preparation except that a greater increase in arterial pressure was observed in the present study. In every animal, marked improvement in cardiovascular function occurred before any change in aldosterone production was observed. One hour after administration of digoxin, at a time when striking hemodynamic improvement was manifest in all animals, the decrease in aldosterone secretion was insignificant in two dogs, moderate in one dog, and maximal in only two of the five dogs. In three of the five dogs, the maximum decrease in aldosterone output did not occur until approximately two hours after the initial improvement in cardiovascular hemodynamics had been observed. Similar results were obtained from the dog with naturally occurring congestive heart failure. Although definite hemodynamic improvement occurred within five minutes after the ouabain injection was begun, minimal aldosterone output was not observed until 80 minutes after the glycoside injection.

In the two dogs with combined tricuspid insufficiency and pulmonic stenosis, no improvement in cardiovascular function was observed following digitalization. The increase in RAP following digitalization apparently resulted from more forceful contraction of the right ventricle in the presence of a fixed degree of pulmonic stenosis and in the absence of the tricuspid valve. In marked contrast to the findings in the dogs with pure
pulmonic stenosis, digoxin administration had no effect on aldosterone secretion in either of the dogs with combined tricuspid insufficiency and pulmonic stenosis. In the present study, a decrease in aldosterone output was observed following digitalization only in those animals in which the glycoside administration resulted in a definite improvement in hemodynamic function, and in these animals, the maximal decrease in aldosterone output did not occur until one to two hours after the changes in cardiovascular hemodynamics had been initiated. Furthermore, a subsequent marked increase in aldosterone secretion was invariably observed in the digitalized dogs in which cardiac decompensation was again produced by further constriction of the pulmonary artery ligatures. These results in dogs with right heart failure appear to differ from the findings of Gomall and Sherrod which suggest that cardiac glycosides may act directly on the adrenal cortex to reduce aldosterone secretion in normal dogs. The probable explanation for the findings of these investigators is that the decrease in aldosterone output in their experiments resulted from the very marked fall in adrenal blood flow following the administration of acetylstrophanthidin to normal animals.

The question remains as to the specific hemodynamic alteration which leads to an increase in aldosterone secretion in right heart failure; and conversely, what functional change results in a decrease in aldosterone secretion as cardiac compensation is restored by cardiac glycosides. Since it has been firmly established that an aldosterone stimulating hormone (ASH) is produced by the kidney, and recent evidence suggests that ASH is either renin or a renin-like substance, it might be suggested that aldosterone secretion is regulated by alterations in renal blood flow. However, the recent finding (and personal observations) that aldosterone secretion is within normal limits in the majority of dogs in the acute phase of benign experimental renal hypertension, in which the renal blood flow is almost invariably reduced, indicates that a decrease in renal blood flow alone does not provide the stimulus necessary to increase aldosterone output. The observation that both hyperplasia and hypergranulation of the renal juxtaglomerular (JG) cells occur in animals with hyperaldosteronism secondary to thoracic caval constriction or to Na depletion points to the JG cells as the site of origin of ASH. These findings are consistent with the hypothesis advanced by Tobian that the JG cells, located in the media of the renal afferent arteriole, act as sensitive receptors which respond to diminished stretch in the afferent arteriolar wall by an increase in secretion of renin. The possibility is thus suggested that decreases in pressure or volume in the renal afferent arteriole, not necessarily dependent upon changes in renal blood flow, may result in an increase in the rate of release of ASH by the JG cells, and thus lead to an increased aldosterone output. The decrease in aldosterone production observed after the administration of cardiac glycosides to animals with right heart failure could then result from increased pressure or volume in the renal afferent arteriole secondary to the improvement in hemodynamic function.

Summary

A marked elevation in the rate of aldosterone secretion was observed in eight dogs with experimental congestive heart failure secondary either to controlled progressive pulmonic stenosis or to combined tricuspid insufficiency and pulmonic stenosis, and in one dog with spontaneously occurring congestive heart failure. In the dogs with pulmonic stenosis, digitalization with digoxin resulted in improvement in cardiovascular function, and subsequent further constriction of the pulmonary artery again led to right ventricular failure; in each animal aldosterone output decreased within 90 minutes after digitalization, and increased markedly within one hour after further constriction of the pulmonary artery. In the dog with naturally occurring congestive heart failure, ouabain administration caused a similar striking hemodynamic...
improvement, and a marked reduction in aldosterone secretion was observed within 55 minutes after the glycoside was given. In the dogs with combined tricuspid insufficiency and pulmonic stenosis, no hemodynamic improvement occurred after digitalization; in these animals, the aldosterone secretion rate did not change significantly during the two-hour period following the digoxin injection. These data demonstrate that changes in the rate of aldosterone secretion in dogs with right heart failure were closely correlated with alterations in cardiovascular function; in these animals, hemodynamic improvement consistently led to decreased aldosterone production, and subsequent worsening of cardiovascular function resulted in increased aldosterone output.

References

22. Davis, J. O., and Ball, W. C., Jr.: Effects of
CARDIAC GLYCOSIDES ON ALDOSTERONE SECRETION

Acute Effects of Cardiac Glycosides on Aldosterone Secretion in Dogs with Hyperaldosteronism Secondary to Chronic Right Heart Failure
Charles C. J. Carpenter, James O. Davis, Charles R. Wallace and William F. Hamilton

doi: 10.1161/01.RES.10.2.178

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1962 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/10/2/178

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation Research_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation Research_ is online at:
http://circres.ahajournals.org/subscriptions/