Effect of Digitalis Glycosides on the Myocardial Sodium and Potassium Balance

By John R. Blackmon, M.D., Herman K. Hellerstein, M.D., Louis Gillespie, Jr., M.D., and Robert M. Berne, M.D.

The effect of nontoxic and of toxic doses of digitalis glycosides on the sodium and potassium balance of the myocardium continues to be disputed despite the development of new technics of investigation. Since 1930, the majority of investigators have found that potassium is liberated from cardiac muscle following digitalis administration. Boyer and Poindexter found an increase in intracellular potassium in the heart muscle after digitalization. Other investigators, however, noted no change in the myocardial potassium and/or sodium concentration after the administration of digitalis.

In the past several years, the technic of coronary sinus catheterization has been used to study the effects of digitalis glycosides in the anesthetized intact dog and in man. The A-V difference of sodium and potassium has been used as a measure of the myocardial exchange. Since the magnitude of the coronary arteriovenous potassium difference depends on (1) the coronary flow per unit of time, (2) the speed with which potassium is released from the heart muscle, and (3) the total potassium lost from the heart, the A-V difference alone may give misleading information about the potassium balance. The conflict of the results of such studies may arise in part from the failure to measure the total amount of potassium lost from the heart.

The purposes of the present study are: (1) to determine the effects of nontoxic and toxic doses of digitalis glycosides on the sodium and potassium balance of the dog heart, (2) to relate the magnitude of the shift and of the cumulative debt of potassium to the onset of cardiac arrhythmias, and (3) to determine the effects of altering the serum potassium level by glucose and insulin and by potassium chloride prior to or following digitalization.

Methods

The experiments were performed on 18 mongrel dogs weighing 15 to 26 Kg. They were anesthetized with intravenously administered sodium pentobarbital (30 mg./Kg.). The chest was opened at the fourth left intercostal space, and respiration was maintained artificially. Heparin (5 mg./Kg.) was injected intravenously to prevent clotting. The aorta was cannulated via the right carotid artery, and phasic aortic pressure was recorded by a modified Gregg manometer. A modified Morawitz cannula was placed in the coronary sinus through a small incision in the right atrial appendage. Coronary sinus blood flow was measured by the method described by Levy and Franke. A Sanborn direct writing apparatus was used to record electrocardiograms (lead II) during the control and experimental periods. Blood samples were drawn at 1, 3, 5, and 10 minute intervals from the coronary sinus and from an indwelling cannula in the femoral artery for determination of plasma sodium and potassium levels. An Internal Lithium Standard Flame Photometer was used to analyze plasma samples in duplicate.

Aortic pressure, heart rate, coronary sinus blood flow, electrocardiograms, and samples of femoral arterial and coronary sinus blood for determining plasma sodium and potassium were obtained during the control and experimental periods.

The effects of digitalis glycosides, glucose and insulin, and potassium chloride on the above parameters were measured. The cardiac glycosides used were Acetyl Strophanthidin (Lilly), 0.5 mg./cc, 4 cat units, and K-Strophosid (Sandoz), 0.5 mg./cc, 3 cat units. The nontoxic dose used was 0.05 mg./Kg., and the toxic dose used with both drugs was 0.1 mg./Kg., given in a single injection or multiple smaller injections. In certain experiments 50 ml. of 50 per cent glucose in water and 15 units of crystalline insulin were administered prior to, together with, or after the digitalis.

*We are indebted to the Eli Lilly Company for the generous supply of Acetyl Strophanthidin.
Table 1

Effects of a Single Toxic Dose of Acetyl Strophantidin on Average Coronary A-V Difference of Sodium and Potassium of Four Dogs (discussed in text)

<table>
<thead>
<tr>
<th></th>
<th>Sodium A-V Difference</th>
<th>S. D.</th>
<th>Potassium A-V Difference</th>
<th>S. D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-4.5 ± 2.04</td>
<td></td>
<td>-0.139 ± 0.021</td>
<td></td>
</tr>
<tr>
<td>After single toxic dose of digitalis glycoside-Acetyl Strophanthidin, 0.1 mg./Kg.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 minute</td>
<td>-5.7 ± 1.70</td>
<td>-0.66 ± 0.042</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 minutes</td>
<td>-3.5 ± 1.80</td>
<td>-1.42 ± 0.145</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 minutes</td>
<td>-2.8 ± 1.92</td>
<td>-1.13 ± 0.173</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average for first 5 minutes</td>
<td>-4.1 ± 1.86</td>
<td>-1.10 ± 0.095</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Glycosides. Varying amounts of a potassium chloride solution (2 mEq./ml.) were given in one series of experiments. All drugs were given via the intravenous route.

In Part I, 9 dogs (nos. 1-9) were given digitalis glycosides and studied until death or the end of the experimental period. In Part II, glucose and insulin were administered to 9 dogs (nos. 6-14) prior to, simultaneously with, or after digitalization. In Part III, 4 dogs (nos. 15-18) were given potassium chloride in varying amounts (60 to 120 mEq.) 40 to 60 minutes prior to digitalization.

Results

Part I: Electrolyte, Hemodynamic, and Electrocardiographic Changes following the Administration of Digitalis

Nine dogs were given a digitalis glycoside at variable times and in variable doses and were observed until death or the end of the experimental period. Five dogs (nos. 1-5) were studied during periods of 70 to 110 minutes, and 4 dogs (nos. 6-9) were studied during periods of 21 to 40 minutes prior to the administration of glucose and insulin. The entire former group and dog no. 9 received Acetyl Strophanthidin while the remainder of the latter group received K-Strophosid. Four dogs (nos. 1, 3, 4, 5) were given single toxic doses; 5 dogs (nos. 2, 6, 7, 8, 9) received multiple nontoxic doses. Four of the first 5 dogs survived the total experimental period which ranged from 70 to 110 minutes with an average of 84 minutes. One dog developed ventricular fibrillation at 70 minutes. The survival times and fates of dogs nos. 6-9 are discussed in Part II.

A biphasic change in potassium levels was observed in all 8 animals followed for more than 35 minutes (fig. 1C). Following intravenous injection of Acetyl Strophanthidin the coronary sinus potassium levels showed an initial sharp rise with a subsequent fall but never returned to control levels. The femoral arterial potassium levels increased gradually, reaching constant levels near the end of the experimental period. There was a significant and consistent negative A-V difference of potassium during the first 25 minutes of the experiments, at the end of which time a positive A-V difference of potassium occurred and continued to the conclusion of the experiments. The peak potassium A-V differences in the 4 dogs given single toxic doses of digitalis occurred in the first 5 minutes (table 1). They averaged 1.10 mEq./liter and ranged from 0.93 to 2.13 mEq./liter. The peak A-V differences in the 5 dogs given nontoxic doses of digitalis also occurred in the first 5 minutes but were of a smaller magnitude, ranging from 0.11 mEq./liter to 0.79 mEq./liter with an average of 0.42 mEq./liter. The average control levels of potassium were 3.0 mEq./liter in both the femoral artery and coronary sinus. The average potassium level at the junction of the femoral arterial and coronary sinus potassium concentration curves was 4.8 mEq./liter. The product of coronary blood flow (CBF) and the A-V difference of potassium also demonstrated the initial negative and later positive myocardial potassium (fig. 1D) balance. There was no consistent or significant alteration in either femoral arterial or coronary sinus sodium levels in the above dogs (table 1).

Following the administration of digitalis there was an initial increase in systemic blood pressure and coronary blood flow lasting 10 to 20 minutes, after which they fell to control or below control values (fig. 1A and B). The changes in heart rate were more variable. In the majority of the dogs the heart rate...
tration of digitalis, the heart rate decreased during the first 3 minutes and increased or remained the same subsequently.

Cumulative Myocardial Potassium Debt and Electrocardiographic Changes

In 8 of 9 dogs the myocardium went into potassium debt for the entire test period. In the one exception (dog no. 1), the potassium debt was repaid, and a small positive balance occurred. The magnitude of the debt was greatest at the 20 to 25 minute period, and became progressively less as the experiment proceeded. An example is shown in figure 1E. The electrocardiographic changes occurring at various times throughout the experiment are shown in figure 2. The letters in figure 1E showing the time sequence and potassium status of the myocardium correspond to the lettering of the electrocardiograms in figure 2.

In this series of experiments the electrocardiographic changes following digitalis administration were typical of digitalis intoxication. The early minor changes, such as slowing of the rate, peaking the T waves, ST-T depression, first degree A-V block, progressed to later and more complex arrhythmias terminating in ventricular asystole and/or fibrillation. The progressive deterioration of the electrocardiograms occurred while the potassium debt was being repaid, and fatal arrhythmias occurred when the debt was almost repaid in toto. Examples are shown in figures 1E and 2.

Part II: Effects of Decreasing Serum Potassium with Insulin and Glucose on Digitalis Effects

In this series of experiments, 9 dogs (nos. 6-14) were given glucose and insulin prior to, simultaneously with, or after digitalis administration. Dogs nos. 6, 7, and 8 were given K-Strophosid, and dogs nos. 9, 10, 11, 12, 13, and 14 were given Acetyl Strophanthidin. All animals were observed until death occurred or to the end of the experimental period.

Glucose and Insulin Prior to Digitalis

Dogs nos. 12, 13, and 14 were given glucose and insulin 30 to 40 minutes prior to the administration of digitalis. These 3 dogs died of ventricular fibrillation 25, 23, and 19 minutes, respectively, after the administration of digitalis. Their control potassium levels were lower than in normal animals treated with digitalis alone. In dog no. 12 the control potassium value in the coronary sinus was 1.94 mEq./liter, in the femoral artery 2.21 mEq./liter; in dog no. 13 control value in the coronary sinus was 1.48 mEq./liter and the femoral artery 1.49 mEq./liter; and in dog no. 14 the control coronary sinus and femoral arterial samples were identical, 1.74 mEq./liter.

Following digitalization, there was no significant A-V potassium difference although in both the coronary sinus and the femoral artery, the potassium rose sharply in the first 5 minutes (fig. 3C).

The circulatory hemodynamics changed markedly. The heart rate increased immediately and remained elevated until death. The blood pressure rose initially but at the end of 10 minutes was below control levels. Coronary blood flow increased 3- to 4-fold and began to decrease at the 15 minute interval but never below control values until death occurred. Figure 3A and B is illustrative of the changes in these 3 animals. The myocardium, which was in positive potassium balance before the digitalis was administered, remained in positive potassium balance in this series of experiments, although fluctuations in the A-V differences occurred after digitalis. The product of the A-V potassium difference times the coronary blood flow showed an initial positive balance followed by a small negative balance with a final positive balance (fig. 3D). The electrocardiographic changes in this series of animals were similar to those in Part I except for the more rapid deterioration of the electrical events terminating in ventricular fibrillation. Figure 4 serves as an example.

Glucose and Insulin Simultaneously with and Following Digitalis Administration

The duration of this experiment was brief, from 3 to 15 minutes. All 6 dogs died of ventricular fibrillation before the end of the experimental period. Dogs nos. 10 and 11 received the drugs simultaneously and died of ventricular fibrillation in 10 and 16 min-
Effects of Acetyl Strophanthidin on the myocardial potassium balance of a dog. In A, vertical bars represent blood pressure and joined dots, heart rate; B, coronary blood flow; C, plasma potassium, in coronary sinus, dots joined by solid lines, and in femoral artery, dots joined by dashes; D, myocardial potassium shift; E, cumulative potassium debt. Arrow indicates intravenous administration of 1.6 mg. of Acetyl Strophanthidin. Note biphasic shift in myocardial potassium, negative uates, respectively. Dog no. 6 was given glucose and insulin 21 minutes after digitalis and died of ventricular fibrillation in 5 minutes. Dog no. 7 was given glucose and insulin 35 minutes after digitalis and died of ventricular fibrillation in 3 minutes. Dog no. 8 was given glucose and insulin 40 minutes after digitalis and died of ventricular fibrillation in 8 minutes. Dog no. 9 was given glucose and insulin 37 minutes after digitalis and died of ventricular fibrillation in 15 minutes. The average survival time in animals given both digitalis and glucose and insulin (including dogs nos. 12, 13, and 14, previously described) was 12.7 minutes.

The electrocardiograms of the animals in this series showed a more rapid progression of events described above, leading to ventricular fibrillation following the administration of glucose and insulin. No other data were obtained in this series of animals because of the rapid onset of death.

Part III: Altering Serum Potassium by Administration of Potassium Chloride

Four dogs (nos. 15-18) were given potassium chloride in varying amounts (60-120 mEq.) prior to but not after the administration of digitalis. Dogs nos. 15 and 18 were given Acetyl Strophanthidin, 0.1 mg./Kg., and dogs nos. 16 and 17 were given K-Strophosid, 0.1 mg./Kg. Dogs nos. 15 and 16 were given 2 doses of digitalis. This series of animals was followed for a total period of 60 minutes with the exception of dog no. 15 which died of ventricular fibrillation 3 minutes after the second dose of digitalis.

The control potassium levels in this series of experiments were much higher, and the coronary sinus and femoral artery samples varied more than in Parts I and II. The average control potassium value in the coronary

for the first 25 minutes and positive thereafter. The cumulative myocardial potassium debt was greatest at 20 minutes and decreased progressively thereafter. Progressive deterioration of the electrocardiogram occurred (see fig. 2) as the debt was reduced, and ventricular fibrillation occurred when positive myocardial balance was attained (discussed in text).
Figure 2

Same dog as figure 1. A shows the control record, lead II; B shows a slowing of the rate, first degree A-V block, and minor S-T depression; C and D show incomplete and complete bundle branch block, respectively; E shows bundle branch block and S-T segment depression; F shows heterotopic activity with periods of bigeminy; G and H show runs of ventricular ectopic beats and complete A-V dissociation; I and J show heterotopic activity with slower rate of the S-A node; K and L show runs of ventricular tachycardia which terminate in ventricular fibrillation (discussed in text).

Sinus of 4 animals was 7.92 mEq./liter. The range in the coronary sinus was 6.9 to 9.4 mEq./liter, and in the femoral artery 8.64 to 10.50 mEq./liter. After the administration of a digitalis glycoside a biphasic change in potassium levels in the coronary sinus and femoral arterial samples was evident even though the levels of potassium decreased in both the coronary sinus and femoral artery as a result of discontinuing the potassium chloride infusion. There was an initial negative A-V potassium balance followed by a small positive A-V potassium balance (fig. 5C).

The heart rate, blood pressure, and coronary blood flow in this series of animals showed the same initial increments after digitalis as in the other experiments. However, the pulse rate remained stable and did not parallel the changes in the levels of the coronary sinus potassium. The blood pressure did not fall much below control levels, and the coronary blood flow returned approximately to control levels. An example of these changes is shown in figure 5A and B.

A marked cumulative potassium debt occurred, and, unlike the other experiments, was not repaid (fig. 5E).

In 3 dogs, minimal electrocardiographic changes occurred. In the control period, the T waves were peaked, reflecting the effect of increased potassium. After the administration of the digitalis glycoside, transient ST-T changes of digitalis effect appeared. No arrhythmias occurred (fig. 6). As mentioned before, dog no. 15 which had shown similar electrocardiographic changes after digitalis, developed ventricular fibrillation 3 minutes after a second dose of digitalis.

Sodium Changes

There were no significant or consistent changes in sodium balance following the administration of the digitalis glycosides (table...
Effect of Acetyl Strophanthidin on myocardial potassium balance of dog treated 30 minutes previously with 50 ml. of 50 per cent glucose and 15 units of crystalline insulin. Legends as before. Arrow indicates intravenous administration of 1.8 mg. of Acetyl Strophanthidin. Note low levels of potassium in both coronary sinus and femoral artery prior to digitalis and the increase of potassium without a significant A-V difference in the first 5 minutes following digitalization. The myocardium remained in positive potassium balance until early death by ventricular fibrillation occurred at 23 minutes (see fig. 4j discussed in text).

Discussion

It is now well established that nontoxic and, especially, toxic amounts of cardiac glycosides influence the electrolyte balance of the heart, but the relation of alterations of myocardial and extracellular potassium to the chronotropic (arrhythmic) action of digitalis has not yet been clarified. Following the administration of a rapidly acting digitalis glycoside, such as Acetyl Strophanthidin, the heart soon develops a negative potassium balance. In the present study, the biphasic shift of potassium of the myocardium, negative for approximately 25 minutes, and then positive, is similar to that also reported in dogs by Hellens and associates.8 In our experiments, this biphasic response was more marked after toxic doses of digitalis glycosides than either after smaller doses or in the potassium pretreatment experiments. When the loss of potassium from the myocardium is extensive and rapid, the coronary arteriovenous difference is great, and myocardial potassium balance is more readily determined. Thus the failure of other investigators to confirm this negative myocardial balance in a similar type of experiments in dog5 or in man7 may be due to the use of other digitalis glycosides, K-Strophosid and Cedilanid, respectively, which produce a slower rate of loss of potassium from the myocardium.

The shifts of potassium produced by digitalis are related to changes produced in the membrane of the myocardial fibers.10 Since there was no consistent relationship between the potassium balance and heart rate in our experiments, it is probable that digitalis produced the imbalance in some other way, either by blocking the re-entry of K+ during dias...
tole or by exaggerating the release of K+ with each systole.

In addition to its action on the myocardium, digitalis glycoside liberates sufficient potassium from extracardiac tissues, such as liver, skeletal muscle, and red blood cells to produce a rise in arterial potassium. In the present and other reports the early rise in coronary sinus potassium is greater than that of the femoral artery, suggesting that the negative balance of the myocardium occurs more rapidly than the mean negative balance of noneardiac tissues. During the subsequent positive balance of the myocardium, the level of the arterial potassium continues to rise, representing continuation of a negative balance of extracardiac tissues.

The disturbance of potassium balance produced by nontoxic doses of digitalis glycosides subsided within an hour, and the myocardial potassium returned to control levels in our study. This finding is in agreement with earlier studies that therapeutic doses did not influence the concentration of potassium in the heart muscle.

The severity of the electrocardiographic changes did not correlate with the magnitude of the cumulative potassium debt, as determined from the coronary arteriovenous difference and coronary blood flow per unit of time. The most serious arrhythmias developed when the myocardial potassium debt had been substantially reduced, and ventricular fibrillation occurred when a positive myocardial potassium balance was attained or was being approached.

The explanation of the above phenomenon is not known, but according to current views should be related to the ionic balances, particularly of potassium between the inside and the outside of the myocardial fibers. The precipitation of fatal ventricular arrhythmias at the time that the potassium balance is being restored suggests the possibilities (1) that the intracellular potassium has not been restored to its predigitalis state but is bound or not readily available for transmembrane release because of the influence of subcellular structures upon the kinetics of potassium

Figure 4
Same dog as figure 3. Electrocardiographic changes (lead II) produced by digitalis glycoside in dog pretreated with insulin and glucose. A and B show inverted T waves 30 to 35 minutes after glucose and insulin administration. B was recorded just before intravenous administration of 1.5 mg. Acetyl Strophanthidin; C, 5 minutes later, shows 8-T segment depression, first degree A-V block, and runs of ventricular premature beats; D, at 10 minutes, shows marked S-T depression and bizarre ventricular conduction; E and F, at 15 and 20 minutes, show complete A-V dissociation and delayed interventricular conduction; G, at 35 minutes, shows ventricular tachycardia terminating in ventricular fibrillation (discussed in text).

within the myocardial fiber, or (2) that the returning potassium is actually situated in the interstitial spaces, and that a potassium balance has not been attained. A more direct approach involving chemical analysis of the myocardium will be required to explain the above finding.

The increased digitalis sensitivity following or accompanying insulin and glucose likewise may be related to a "modified state" of intracellular potassium as well as to low serum potassium. It has been amply demonstrated that several inorganic substances including potassium participate in carbohydrate metab-
Effects of Acetyl Strophanthidin on myocardial potassium balance of dog pretreated with potassium chloride. Legends as before. Sixty mEq. of potassium chloride were administered intravenously during 35 minutes prior to the administration of 1.75 mg. of Acetyl Strophanthidin, indicated by arrow. Note biphasic shift in myocardial potassium balance, negative for 20 minutes, and positive later. Although the cumulative myocardial potassium debt increased, no arrhythmias occurred (see fig. 6; discussed in text).
ing either that the potassium was liberated primarily from extracardiac tissues, or that the usual potassium efflux produced by digitalis was approximately equal to an influx secondary to the effects of insulin on the myocardial membrane.

The development of early death in the insulin and glucose pretreated animals is even more difficult to explain, since ventricular fibrillation occurred when the potassium levels in the coronary (femoral) arterial and venous circulation (Ko+) were restored to normal or above normal, that is, the predigitalis hypokalemia had subsided, and the myocardium was in positive potassium balance. This differs from the digitalis sensitivity in patients following carbohydrate administration or during dialysis which is related predominantly to the low extracellular potassium levels. The lethal effect of a combination of digitalis, insulin and glucose, the relationship between the electrocardiographic changes and carbohydrate meals in digitalized patients, and the hypokalemia in insulin-induced hypoglycemia have obvious clinical importance.

The protective action of potassium salts against digitalis intoxication first noted by Sampson and Anderson in 1930 has been confirmed by the present data and by other investigators. In our experiments, pretreatment with potassium undoubtedly increased both the intracellular and extracellular concentration of potassium ions and protected the heart from the toxic effects of digitalis glycosides. The increase of extracellular potassium ions has an effect opposite to those of decreasing extracellular potassium (see above). The animals pretreated with potassium showed effects opposite from the insulin and glucose pretreated group, namely, in the former the serum potassium was elevated, and T waves were peaked; and after the administration of digitalis despite a biphasic myocardial potassium shift, only minor electrocardiographic changes occurred. These disappeared before the termination of the experiment in contrast to the fatal and rapid progression of the insulin and glucose experiments. It is possible that in the insulin and glucose pretreated dogs Ko+ did not rise sufficiently to reverse the toxic actions of digitalis.

In the present study, as in that of others, no consistent or significant changes in the concentration of sodium were detected. Hellens and associates, however, did report a sodium shift and a change in pH.

In our study, changes in coronary blood flow correlated with the systemic blood pressure. Both increased immediately after digitalization and decreased when arrhythmias appeared. These changes are probably a function of cardiac output and may suggest that some degree of occult cardiac failure was present in our preparations. Other investigators have found that doses comparable to those used clinically produce variable effects on blood pressure and either no significant change or a small decrease in coronary blood flow.

Summary

Nontoxic and toxic doses of digitalis glycoside produced a rapid release of potassium from the myocardium and from extracardiac tissues of the dog. For approximately 25 minutes after the administration of a digitalis glycoside the myocardium was in negative potassium balance and within an hour returned to positive balance. The most serious electrocardiographic changes transpired when the myocardium approached or attained potassium balance. Pretreatment with potassium chloride protected the heart against arrhythmias by increasing Ko+. Pretreatment with glucose and insulin enhanced the arrhythmic action of the digitalis glycosides presumably by producing a low serum potassium (Ko+) and possibly by modifying the state of intracellular potassium (Ki+).

Acknowledgment

We are indebted to Dr. Nick Sperelakis, Department of Physiology, School of Medicine, Western Reserve University, for helpful comments and criticism.

Summario in Interlingua

Doses toxica e nontoxica de glycoside de digitalis producva un rapide liberation de kalium ab le myocardio e ab tissus extracardiac del can. Durante
alterationes electrocardiographicae occurrentes quando le myocardio appropinquava o attingeva lo stato de balanci di calium. Pretrectamento con chloruro de kalium protegeva le corde contra arrhythmias per augmentar le K⁺ extra le fibras myocardiali. Pretrectamento con glucosa e insulina promoveva le effecto arrhythmemic del glycosidos de digitalis, presumitementemente per producer cosido de digitalis, le niyocardio se trovava in circa 25 minutas post le administration do un glycosido de kalium. Pretrectamento con chloruro de kalium le lv + extra le fibras myocardial. Pretrectamento con negative balancia de kalium. Intra un hora illo glucosa e insulina promoveva le effecto arrhythmemic del electrocardiograms, presumitemente per modificar le stato de kalium intracellular.

References


Circulation Research, Volume VIII, September 1960
Effect of Digitalis Glycosides on the Myocardial Sodium and Potassium Balance
JOHN R. BLACKMON, HERMAN K. HELLERSTEIN, LOUIS GILLESPIE, JR. and ROBERT M. BERNE

Circ Res. 1960;8:1003-1012
doi: 10.1161/01.RES.8.5.1003
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1960 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/8/5/1003

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/