A Comparison of the Serum Lipids, Lipoproteins, Glycoproteins, Urinary 17-Ketosteroids, and Gonadotropins in Eunuchs and Control Male Subjects


The proclivity of men to coronary artery disease has been recognized for almost 2 centuries. The gonadal hormones have understandably come under scrutiny in the search for the explanation of this marked sex difference in susceptibility to disease. Sex hormones are known to alter the physical state of the serum lipids. It has been suggested that eunuchs suffer a less severe form of coronary atherosclerosis than uncastrated subjects. This study compares the serum lipids and lipoproteins, as well as other serum and urinary constituents, in 24 castrated and 20 uncastrated institutionalized men.

The concept of coronary atherosclerosis primarily as a disease of men had its origin almost 2 centuries ago and appears to be one of the few points over which there is relatively little disagreement among students of atherosclerosis. Indirect support of this concept is provided by Wuest, Dry, and Edwards\(^2\) and by Rivin and Dimitroff\(^3\) whose studies indicated that castrated women suffer a more severe form of coronary atherosclerosis than uncastrated women. More directly applicable are the suggestions of White\(^4\), Howard and Gertler,\(^5\) and Hawke\(^6\) that eunuchs manifest less anatomic evidence of coronary atherosclerosis than uncastrated men. These differences may be related, at least in part, to the known influence of gonadal steroids on serum lipids and lipoproteins.\(^7\) Considerations such as these prompted us to study a group of 24 eunuchs and 20 uncastrated men, all institutionalized at a training school for the mentally retarded.

Selection of Subjects and Methodology

Only subjects in apparent good health were retained in this study after physical examination, electrocardiogram, chest x-ray, and determinations of the serum total protein and hemoglobin had been made. There were no hypertensive individuals or members of the Hebrew race. There was 1 Negro among the eunuchs, none in the control group.

The age distribution for both groups was between 23 and 79 years and is shown in figure 1. The mean age of the eunuchs was 39, that of the controls 41. Castrated and control subjects were matched with respect to age and weight as closely as the nature of the school population would permit.

The mean body weight of the castrates was 69.5 ± 2.7 Kg., that of the controls 65.5 ± 2.3 Kg. Mean body surface area of the castrates was 1.80 ± .04 M\(^2\), that of the controls 1.72 ± .03 M\(^2\).

From the Cardiovascular Section and the Endocrinology and Metabolism Section, Oklahoma Medical Research Foundation, the Departments of Medicine and Biochemistry, University of Oklahoma School of Medicine and the Research Laboratory, V. A. Hospital, Oklahoma City, Okla.

This study was supported in part by research grants from the National Heart Institute (H-1429 and H-1694) of the U. S. Public Health Service, the Oklahoma State Heart Association, The Smith, Kline and French Foundation, Philadelphia, Pa. and the Ayerst Laboratories, New York, N. Y.
The mean age at castration was 18 years. The relationship between age at castration and age at the time of this study is depicted in figure 2. It is of interest to note that castration had been accomplished by the sixteenth year in 10 subjects. One subject, age 28, was found on examination...
<table>
<thead>
<tr>
<th>Determination</th>
<th>Age</th>
<th>Castrates</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean values</td>
<td>No. subjects</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>21-30†</td>
<td>161±6</td>
<td>7</td>
</tr>
<tr>
<td>mg. %</td>
<td>31-50</td>
<td>180±9</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>51 &amp; over</td>
<td>182±2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>All ages</td>
<td>175±6</td>
<td>24</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>21-30</td>
<td>226±8</td>
<td>7</td>
</tr>
<tr>
<td>mg. %</td>
<td>31-50</td>
<td>238±8</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>51 &amp; over</td>
<td>243±3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>All ages</td>
<td>235±5</td>
<td>24</td>
</tr>
<tr>
<td>Ratio:</td>
<td>21-30</td>
<td>0.70±0.03</td>
<td>7</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>31-50</td>
<td>0.72±0.02</td>
<td>13</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>51 &amp; over</td>
<td>0.77±0.03</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>All ages</td>
<td>0.73±0.01</td>
<td>24</td>
</tr>
<tr>
<td>−S₁₂₁ 0-12</td>
<td>21-30</td>
<td>207±11</td>
<td>7</td>
</tr>
<tr>
<td>mg. %</td>
<td>31-50</td>
<td>195±10</td>
<td>13</td>
</tr>
<tr>
<td>(&quot;alpha&quot;)</td>
<td>51 &amp; over</td>
<td>217±4</td>
<td>4</td>
</tr>
<tr>
<td>lipoproteins</td>
<td>All ages</td>
<td>202±7</td>
<td>24</td>
</tr>
<tr>
<td>−S₁₂₁ 25-40</td>
<td>21-30</td>
<td>173±6</td>
<td>7</td>
</tr>
<tr>
<td>mg. %</td>
<td>31-50</td>
<td>183±14</td>
<td>13</td>
</tr>
<tr>
<td>(&quot;beta&quot;)</td>
<td>51 &amp; over</td>
<td>191±16</td>
<td>4</td>
</tr>
<tr>
<td>lipoproteins</td>
<td>All ages</td>
<td>182±8</td>
<td>24</td>
</tr>
<tr>
<td>−S₁₂₁ 25-70</td>
<td>21-30</td>
<td>203±9</td>
<td>7</td>
</tr>
<tr>
<td>mg. %</td>
<td>31-50</td>
<td>227±13</td>
<td>13</td>
</tr>
<tr>
<td>(&quot;beta&quot;)</td>
<td>51 &amp; over</td>
<td>219±17</td>
<td>4</td>
</tr>
<tr>
<td>lipoproteins</td>
<td>All ages</td>
<td>219±8</td>
<td>24</td>
</tr>
<tr>
<td>Ratio:</td>
<td>21-30</td>
<td>1.21±0.05</td>
<td>7</td>
</tr>
<tr>
<td>−S₁₂₁ 0-12</td>
<td>31-50†</td>
<td>1.17±0.12</td>
<td>13</td>
</tr>
<tr>
<td>(&quot;alpha&quot;)</td>
<td>51 &amp; over</td>
<td>1.19±0.10</td>
<td>4</td>
</tr>
<tr>
<td>(&quot;beta&quot;)</td>
<td>All ages§</td>
<td>1.18±0.07</td>
<td>24</td>
</tr>
<tr>
<td>Ratio:</td>
<td>21-30</td>
<td>1.29±0.05</td>
<td>7</td>
</tr>
<tr>
<td>−S₁₂₁ 25-40</td>
<td>31-50†</td>
<td>1.12±0.07</td>
<td>13</td>
</tr>
<tr>
<td>(&quot;alpha&quot;)</td>
<td>51 &amp; over</td>
<td>1.21±0.03</td>
<td>4</td>
</tr>
<tr>
<td>(&quot;beta&quot;)</td>
<td>All ages†</td>
<td>1.18±0.04</td>
<td>24</td>
</tr>
<tr>
<td>Ratio:</td>
<td>21-30</td>
<td>0.91±0.04</td>
<td>7</td>
</tr>
<tr>
<td>−S₁₂₁ 0-12</td>
<td>31-50</td>
<td>0.81±0.04</td>
<td>13</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>51 &amp; over</td>
<td>0.93±0.05</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>All ages†</td>
<td>0.86±0.03</td>
<td>24</td>
</tr>
</tbody>
</table>

* Values listed are "true means" where N = number of subjects (rather than number of samples) studied.

† Differences between castrates and controls in this group significant at 0.05 level.
‡ Differences between castrates and controls in this group significant at 0.02 level.
§ Differences between castrates and controls in this group significant at 0.001 level.
during the course of the study to be eunuchoidal but not castrate. There was a history of testicular atrophy early in childhood, the testes were extremely small, and the body build, fat, and hair distribution were definitely eunuchoidal. Because of these findings he was retained in the castrate group only for compilation of data presented in the tables.

Five blood samples were obtained from each eunuch (total number of samples 124) and 3 from each control subject (total number 70) in most instances. Blood was usually obtained at intervals of several months over a 2-year period. All blood was drawn in the postabsorpive state. Overnight urine specimens were collected whenever possible for determinations of 17-ketosteroid and urinary gonadotropin. Analyses were performed on a total of 49 urine specimens from 19 control subjects and 67 specimens from 24 castrates.

Lipoproteins were determined refractometrically after preparative ultracentrifugation at solvent density 1.21 Gm. per ml., according to the method of Lewis, Green, and Page. The value of the ratio of high density lipoproteins to low density lipoproteins is employed to provide an index of the relative amounts of these 2 major lipoprotein groups. Use is also made of the values of the ratios of high density lipoproteins to total cholesterol and phospholipid.

Urinary gonadotropin was assayed according to the nondialysis method of Klinefelter, Albright, and Griswold, and levels of less than 96 mouse uterine units per 24 hours were considered normal. Urinary neutral 17-ketosteroids were determined by the Zimmerman reaction. Initially, acid hydrolysis, extraction with carbon tetrachloride and a modified Zimmerman reaction were employed according to the technic outlined by Holtorff and Koch. In analyses undertaken after May 1955, the technic proposed by Klendshoj, Feldstein, and Sprague was employed, without change in values considered “normal.”

Serum glycoprotein (protein-bound carbohydrate) according to the method of Shetlar, Foster, and Everett, in which the results are expressed in terms of bound hexose, and total serum protein by the method of Weichselbaum were determined in 27 samples of serum from 11 control subjects and in 63 samples from 22 castrates. The ratio total protein serum glycoprotein hexose x 100 is utilized to relate the concentration of protein-bound carbohydrate to the total concentration of circulating protein. The mean age of the controls whose sera were studied for glycoproteins was 36 years, that of the castrates 40.

The data for the 2 groups are compared according to the following age classifications: 21-30 (“young”), 31-50 (“middle-aged”), and over 50 years of age (“old”), as well as irrespective of age. The significance of differences was determined by t test.

**RESULTS**

Mean values for several lipid and lipoprotein fractions studied are presented in table 1.

Mean serum total cholesterol levels were significantly lower in the castrates only in the young or 21 to 30-year age group. The mean values for the 2 groups in the other age classifications were not significantly different. The degree of esterification was similar in all of the subjects.

Differences in serum phospholipid concentrations between eunuchs and controls were not significant at any age level, nor did cholesterol/phospholipid ratios differ significantly.

Higher concentrations of high density (−S₁₂₁ 0-12 or “alpha”) lipoproteins characterized the castrate group at each age level as well as irrespective of age, although these differences were not statistically significant.

Lower concentrations of lower density (−S₁₂₁ 25-40 or “beta”) lipoproteins characterized the castrate group at every age level, but statistically these differences were significant only when the castrate and control groups were considered in their entirety. The more inclusive −S₁₂₁ 25-70 lower density lipoprotein concentrations were also consistently lower in the castrates but, as was the case for the −S₁₂₁ 25-40 fraction, the difference was statistically significant only when the 2 groups were considered in their entirety.
Low density lipoproteins with flotation rate characteristics above \(-S_{1.21}\) 70 were inconsistently present in small amounts and did not differ significantly in the 2 groups. Concentrations of \(-S_{1.21}\) 20-25 ("alpha-2") lipoproteins likewise did not differ in the 2 groups.

The values for high density: lower density
\[
\left(\frac{-S_{1.21}}{25-40} \text{ or } \frac{-S_{0.12}}{0-12} \right)
\]
lipoprotein ratios were consistently higher in the castrates for all age groups, and the difference between castrates and controls was statistically highly significant in the middle and old age groups, and for the groups considered in their entirety.

The \(-S_{1.21} 0-12\) and \(-S_{1.21} 0-12\)
ratios had consistently higher values for all ages in the castrate group, but the differences were statistically significant only when the groups were considered in their entirety.

Mean values of glycoprotein and total protein are listed in Table 2. Serum glycoprotein concentrations were significantly higher in the castrate group as was the value for the
\[
\text{glycoprotein hexose } \times 100
\]
Serum glycoprotein levels in the control subjects were generally within "normal limits," as determined on a larger group of apparently healthy subjects by Shetlar, Foster, Kelly, and Everett.\(^{17}\) No significant differences were noted with respect to total serum protein concentrations.
Urinary 17-ketosteroid and gonadotropin values are presented in tables 3 and 4. The 17-ketosteroid excretion per 24 hours did not differ significantly between the 2 groups for any age category. When the urinary 17-ketosteroid concentration was determined, a significantly higher level was noted in the total control group. Urinary gonadotropin excretion was as expected, with the majority of eunuchs (22 out of 24) excreting more than 192 mouse uterine units per day while only 3 of 19 control subjects excreted more than 192 units.

**DISCUSSION**

The relatively small size of the samples of this unique population that were available for study limits the degree of statistical significance that can be assigned to many of the observed differences. Outstanding among the data for the castrates, however, are the consistently higher mean values for high density lipoprotein concentrations and the consistently lower mean concentrations of the lower density lipoproteins. There were no exceptions with respect to these mean values (table 1). The likelihood that such consistency was due to chance alone is extremely small (1.212). The value for the ratio of high density/lower density lipoprotein is, by virtue of its derivation, higher in the castrate group. Further emphasis is lent this consistency of difference in lipoprotein concentrations (and ratios) between the 2 groups by the fact that other than for the significantly lower native serum total cholesterol levels in the young castrates, neither serum cholesterol nor phospholipid values differed significantly or consistently.

The differences between eunuchs and uncastrated men with respect to the high and lower density lipoproteins are characteristic of the changes observed when androgens are withdrawn from, or estrogens administered to, other subjects.7

The significantly lower serum cholesterol levels in the young castrates prompted further scrutiny of the data with reference to what influence the subject's age at the time of castration might have on his lipids and lipoproteins at the time of study. Figures 2 and 3 are of interest in this regard. It may be noted (fig. 2) that none of the young castrates had a serum cholesterol level in excess of the control mean serum cholesterol level. A young age at castration is of course inherent in the young castrate group. When the middle and old age groups are examined, it is of further interest to note that while none of the eunuchs who was subject to castration before the seventeenth year of age had a serum cholesterol value in excess of the mean serum cholesterol value, each of the 4 prepuberal castrates in the middle age group had individual mean serum cholesterol values (137 ± 6, 149 ± 10, 156 ± 3, and 141 ± 14 mg. per cent) statistically significantly lower (p < 0.01, p < 0.05, p < 0.05, p < 0.05) than the mean cholesterol level (180 ± 9 mg. per cent) for the middle age castrate group as a whole. There are, of course, some eunuchs in the middle age group who underwent castration later than the sixteenth year of age whose serum cholesterol values were also less than the mean control value.

Figure 3 denotes a somewhat different relationship among the 3 variables: age at castration, age at time of study, and the value for the ratio high density/lower density lipoproteins. It is noted that none of the values of this ratio in the castrate group was less than the mean control value, irrespective of age at castration or age at the time of study. It is also of interest to note that only 1 control subject, who happened to fall in the young age group, had a value for the high
density/lower density lipoprotein ratio that exceeded the mean value of this ratio in the eunuchs.

These observations suggest that the influence of the male gonad on the relative amounts of high and lower density serum lipoproteins is at least partially independent of age factors, e.g., the age at which gonadal influence is removed (at least up to the age of 33 years), and probably the age of the subject at the time serum lipoprotein relationships are determined, although the data suggest that alterations in lipoprotein relationships induced by gonadectomy are apt to be more striking in middle-aged male subjects. The influence of castration before the seventeenth year of age on the level of chemically determined serum cholesterol also appears to be somewhat independent of age, since the lower level characteristic of the young castrates was noted in the middle age group only in those subjects who were prepuberal castrates.

These data, then, are consistent with the hypothesis that the effect of withdrawal of gonadal hormones on the relative amounts of the 2 major serum lipoprotein fractions in men is at least partially independent of age, while the effect on the cholesterol concentration is maintained only when the gonads are removed at or before puberty.

The lack of significant differences in serum phospholipid levels in the 2 groups, previously noted by Hamilton, Bunch, Mestler and Imagawa,\textsuperscript{18} is undoubtedly related to the fact that the lipid phosphorus content of the high and lower density lipoproteins is similar, namely 26.5 and 23.4 per cent respectively, as reported by Bragdon, Havel, and Boyle.\textsuperscript{19}

The lack of significant differences between the eunuchs and uncastrated men with respect to low density lipoproteins of flotation rates in excess of \(-S_{1.21} 70\) (S\textsubscript{f} 20) tends to minimize the etiologic or predictive significance of these low density lipoproteins in atherosclerosis (assuming that eunuchs suffer less atherosclerosis than uncastrated men).

The lower levels of urinary 17-ketosteroids in the eunuchs are obviously a consequence of castration, although the difference in 17-ketosteroid excretion between the 2 groups is quantitatively slight, presumably because of increased adrenal androgen synthesis in the eunuch. Qualitatively these adrenal androgens differ from those of gonadal origin inasmuch as they do not prevent the appearance of eunuchoidal somatic characteristics in the prepubescent castrate. The difference in the lipoprotein relationships of the 2 groups demonstrated in this study is an additional manifestation of this qualitative difference between testicular and adrenal androgens. No correlation was noted between urinary 17-ketosteroid excretion and a subject's age at castration, the concentration of high or low density lipoproteins or their ratio. Aging appeared to be associated with diminishing urinary 17-ketosteroids, a phenomenon previously described by Venning and Kazmin.\textsuperscript{20}

A greater excretion of gonadotropins in castrated than in uncastrated subjects, is of course, expected. The reasons for elevated gonadotropin excretion in 3 of the control subjects are not apparent, since testicular size and consistency were normal in each subject and there were no other stigmata of eunuchoidism or general systemic disease. Two of them were 53 and 79 years old and thus may have been examples of testicular tubular failure occurring with advancing years. When all data from these 3 subjects were excluded, no significant alteration of any of the mean values in the control group resulted.

Scrutiny of the lipid data for intragroup differences related to age reveals a significant \((p < 0.05)\) increase in lower density \(-S_{1.21} 25-70\) lipoprotein concentrations in the control subjects on going from the young to the middle age group. This is in keeping with the observation of Jones et al.\textsuperscript{21} that greater concentrations of \(S_f 12-20\) \((-S_{1.21} 25-40)\) lipoproteins are noted in men beginning with the third decade of life. The failure of serum cholesterol to increase along with the lower density lipoproteins in this respect is attributable to the reduction in high density lipoprotein levels, which reduction, although
not statistically significant, nevertheless was of sufficient mean magnitude to obviate change in the mean cholesterol concentration on going from the young to middle age control group.

The increase in serum cholesterol levels noted in the eunuchs on going from the young to the middle age group is significant \((p < 0.01)\) and resembles the increase reported to occur in women from age 33 through 58.\(^2\)

The meaning of the glycoprotein and glycoprotein/total protein differences must await further study. At the present time all that can be said is that castrated subjects have higher serum glycoprotein concentrations than uncastrated subjects, absolutely as well as relative to the total serum protein concentration.

Finally the question of the possible relationship of these findings to the suggestion that the castrate enjoys a relative freedom from coronary atherosclerosis must be considered. It is tempting to suggest that the relatively greater concentrations of high density \(-S_{1.21} 0-12\) lipoproteins noted in the castrated group may be importantly related to this phenomenon. Such thinking gains support from the studies of Jencks and co-workers,\(^3\) who demonstrated reduced concentrations of high density "alpha" lipoproteins and increased concentrations of lower density "beta" lipoproteins in men with a history of myocardial infarction. The predictive significance (and, therefore, possibly the etiologic significance) of the lower density \(S_{1} 12-20\) and 20-100 lipoprotein concentrations in coronary atherosclerosis is certainly in doubt at present.\(^4\)

While the correlation between the lipoprotein spectrum (considered in its entirety) and coronary atherosclerosis is receiving increasing support as a result of studies such as these, the establishment of such a correlation of course does not provide proof of etiologic relationship. Further work along these lines is clearly necessary.

The only other significant and sustained alteration in serum constituents reported to follow castration in the young adult male is a rise of 1 to 1.5 mg. in serum inorganic phosphate.\(^6\) The meaning of this rise with respect to atherogenesis remains conjectural at this point.

The possibility must not be overlooked that personality changes resulting from castration are important, as well as the lipid changes. Increased tractability, stability, diminished aggressiveness, and, in general, a more tranquil existence usually follow castration in the human subject.\(^6\) These characteristics tend to be associated in the minds of clinicians with relative freedom from disorders attributable to "stress," and with longevity and health.

**Summary**

Eunuchs and uncastrated male subjects are compared with respect to serum lipids, lipoproteins and glycoproteins, and with respect to the urinary excretion of gonadotropins and 17-ketosteroids.

The mean high density \(-S_{1.21} 0-12\) ("alpha") lipoprotein concentrations were consistently higher, and the lower density \(-S_{1.21} 25-40\) or 25-70 ("beta") lipoprotein concentrations consistently lower, in eunuchs than in uncastrated controls, irrespective of age at the time of study or time of castration. The ratio of "alpha" to "beta" lipoproteins was also consistently higher in eunuchs. These differences are characteristic of androgen withdrawal or estrogen administration.

The mean serum cholesterol level was significantly lower in the young eunuchs than in young controls. Lower individual serum cholesterol values in the middle aged eunuchs were noted only in those who had undergone gonadectomy prior to the seventeenth year of age. Mean serum cholesterol levels of the middle and old age castrated subjects and controls did not differ significantly.

The mean serum glycoprotein concentration was higher in eunuchs than in the controls. This was also true for the ratio of glycoprotein hexose \times 100\) total protein.

Urinary gonadotropin excretion was in excess of 192 mouse uterine units per day in 22 of 24 eunuchs, while only 3 of 19 control
subjects excreted more than 192 units per day.

Urinary 17-ketosteroid excretion per day did not differ significantly between the 2 groups. However, the urinary 17-ketosteroid concentration was significantly higher in the controls.

ACKNOWLEDGMENT

The authors wish to express their sincere thanks to Col. John B. Smith, Superintendent of the Winfield State Training School, Winfield, Kan., for permission to study these subjects, and to Dr. Harold H. Jones, Dr. Franklin R. Miller, Leitha Bunce, M.A., and other members of the Training School Staff at Winfield for much valuable assistance.

Acknowledgment is gratefully made of the technical assistance provided by Jane B. Faulkner, M.S., Alice W. Fryer, B.S., Betty S. Wamaek, B.S., Emma Lou McDearmon, B.S., Marian MacAulay, Helen M. Hoffman, B.S., and Ilse M. Neeland. We wish also to express our thanks to Dr. Carl R. Doering, Professor of Preventive Medicine and Public Health, University of Oklahoma School of Medicine, and to Joanna Peter, B.S., for statistical analyses of the major portion of the data.

SUMMARIO IN INTERLINGUA

Eunuchos e non-castrate subjectos mascule es comparate con respecto al lipidos, lipoproteinas, e glycoproteinas del sero e al excretion urinari de gonadotropinas e 17-ketosteroides.

Le concentrationes medie del lipoproteinas a alte densitate \(-S_{1,21} \leq 0.12\) ("alpha") eseva regularmente plus alte e le concentrationes medie del lipoproteinas a plus basse densitales \(-S_{1,21} \geq 25-50\) o 25-70 ("beta") eseva regularmente plus basse in eunuchos que in le non-castrate subjectos de controlo, sin regardo al etate del individuos al tempore del studio o al tempore del castration. Le proportion de lipoproteinas "alpha" a lipoproteinas "beta" eseva etiam regularmente plus alte in eunuchos. Iste differentias es caracteristic effectos del abstention de androgeno o del administration de estrogeno.

Le nivello medie del cholesterol in le sero eseva significativamente plus basse in juvenile eunuchos que in juvenile subjectos de controlo. Casos individual de reducite valores del cholesterol seral in eunuchos de etates intermedi-

REFERENCES


A Comparison of the Serum Lipids, Lipoproteins, Glycoproteins, Urinary 17-Ketosteroids, and Gonadotropins in Eunuchs and Control Male Subjects

ROBERT H. FURMAN, R. PALMER HOWARD, M. R. SHETLAR, E. CORINNE KEATY and RICHARD IMAGAWA

Circulation. 1958;17:1076-1085
doi: 10.1161/01.CIR.17.6.1076

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1958 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/17/6/1076

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation 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 is online at:
http://circ.ahajournals.org//subscriptions/