The Effects of Gonadectomy on Left Ventricular Function and Cardiac Contractile Proteins in Male and Female Rats

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SUMMARY. To examine the influence of the sex hormones on mechanical properties and biochemistry of the adult heart, we studied left ventricular function and cardiac contractile proteins in hearts from 20-week-old male and female rats that had been gonadectomized at 18 days of age, compared with hearts from sham-operated animals. Testosterone and estradiol were not detectable in serum from male and female gonadectomized rats, respectively. The male rats had lower body and heart weights than male sham-operated rats, whereas these values were higher in female gonadectomized than in female sham-operated rats. Left ventricular function was studied in a working heart apparatus at similar heart rate and at controlled levels of aortic diastolic pressure and left atrial pressure. At moderate left atrial pressure, end-diastolic pressure and volume per gram dry left ventricle were the same in all groups, but at high left atrial pressure, end-diastolic pressure, and volume per gram dry left ventricle were lower in male and female gonadectomized than in the respective sham-operated rats. Further increases in left atrial pressure were associated with mechanical alternans in male and female gonadectomized rats. Significantly (P < 0.05) lower values for cardiac output, peak systolic pressure, ejection fraction, and myocardial oxygen consumption occurred in male gonadectomized compared with sham-operated rats at moderate and high left atrial pressure at higher levels of aortic diastolic pressure. Decreases in these values for female gonadectomized compared with sham-operated rats occurred only at high left atrial pressure. A significant downward shift in the mean force-velocity relationship was observed in all gonadectomized rats at both moderate and high left atrial pressure. In a follow-up study, when end-diastolic pressure was kept the same at both moderate and high left atrial pressure in female sham-operated and gonadectomized rats by reducing heart rate, decreases in contractile function in gonadectomized rats were observed at all preloads. Ca++-myosin ATPase activity was significantly reduced by 34% in male and by 19% in female gonadectomized rats compared to respective sham-operated control hearts. These alterations in myosin ATPase activity were associated with a reduction in the V1 myosin isoenzyme and an increase in the V3 isoenzyme. Thus, left ventricular filling and left ventricular function were impaired in hearts of gonadectomized rats. Alterations in function were associated with depressed cardiac myosin ATPase activity in male and female gonadectomized rats. These findings suggest that the sex hormones have cardioregulatory properties, but it remains uncertain whether their effect is exerted during or after maturation. (Circ Res 54: 38-49, 1984)

SEX differences in cardiovascular responses to a variety of experimental interventions have been documented. These include differential responses to vasoactive agents (Altura, 1975; Baker et al., 1978) and differences in the activity of enzymes associated with cardiac lysosomes and the inner mitochondrial membrane (Koenig et al., 1982). Studies from our laboratory have shown marked sex differences in cardiac adaptations to chronic physical training in rats (Schaible and Scheuer, 1979, 1981; Schaible et al., 1981). Differences in the incidence of coronary artery disease in human males and females have also been well documented (McGill and Stern, 1979). A possible underlying mechanism for these differences is suggested by the recent demonstration of specific receptors for androgens and estrogens in the myocardium of rats (Krieg et al., 1978, 1980; Stumpf et al., 1977) and baboons (McGill and Sheridan, 1981; McGill et al., 1980).

With this background, it is logical to speculate that the sex hormones play a physiological role in cardiac function. However, cardiac function has not been studied under conditions of chronic sex hormone deficiency. Therefore, the purpose of this study was to examine left ventricular (LV) function after gonadectomy in male and female rats. To maximize a possible contributory role of the sex hormones, hearts from adult animals that had been gonadectomized prepuberally were studied.

To assess left ventricular function in the absence of systemic vascular and neurohumoral effects, we employed the isolated working rat heart preparation.
Experimental Groups

Male and female Wistar rats (Charles River) were gonadectomized or sham-operated at 18 days of age. The surgical procedures were performed by the supplier, using the techniques described by Waynforth (1980), and the animals were shipped to our laboratory 1 week after surgery. All animals were fed Purina rat chow and watered ad libitum for the duration of the study. Body weights were measured in all animals on a weekly basis. Isolated heart studies were performed when the animals were 18–20 weeks of age. At the time of sacrifice, adequacy of the gonadectomy was determined grossly by the absence of testicular tissue in male rats and by the absence of ovarian tissue and marked atrophy of the uterus in female rats.

In a separate study, hearts from younger male rats were perfused in order that function of small male hearts in the isolated heart apparatus could be examined. These animals were also of the Wistar strain and were neither gonadectomized nor sham-operated. They were approximately 10 weeks of age at the time of study.

Heart Perfusions

Full descriptions of the isolated working rat heart apparatus have been published previously (Bersohn and Scheuer, 1977; Schaible and Scheuer, 1981). Hearts were removed from ether-anesthetized rats and immersed immediately in 0°C saline. The period of cardioplegia prior to attachment to the perfusion apparatus was 2–3 minutes. The perfusate was a modified Krebs-Henseleit buffer at 37°C gassed with a 95% O2-5% CO2 mixture and contained 15 mM glucose, 0.01 U/ml regular insulin (Lilly), and 2.0 mM calcium with 0.5 mM EDTA yielding 1.5 mM free calcium. The perfusate was not recirculated. Left ventricular pressure was measured through a 2.5-cm polyethylen (PE-60) catheter inserted through the apex of the heart and attached to a Statham P23d strain gauge pressure transducer. A second catheter (PE-20) was placed through the LV apex for dye injection. Aortic pressure was measured from a sidearm on the aortic cannula approximately 7–8 mm above the aortic valve. The frequency responses of the pressure measuring systems were flat ± 10% to 30 Hz. This frequency response is not sufficient to measure dP/dt accurately (less than 5% error), but this can account only for the absence of differences between experimental groups. Instantaneous aortic flow was measured from a cannulating 2.5 mm i.d. flow probe (Statham-Gould, frequency response set to 50 Hz) inserted in the aortic outflow tubing. Coronary flow was measured directly as right heart outflow, and cardiac output was measured as aortic flow plus coronary flow. Dye concentrations in the aorta were measured by a densitometer system placed into the aortic cannula. All hearts were paced from the right atrium at a rate of 340 beats/min. Oxygen tension was measured in the perfusate from the left atrial reservoir and from a pulmonary arterial catheter.
and dye injection were positioned, antegrade perfusion was begun by unclamping the left atrial catheter. Patency of the catheter in the left atrium was confirmed by the observation of an immediate bulging of the left atrial appendage. After a 10-minute equilibration period, in which the pressure transducers and flow probe were zeroed, measurements of dynamics were made at the moderate and high LAP at an ADP of 59 mm Hg. Measurements at each LAP were then made at an ADP of 81 mm Hg and finally at an ADP of 103 mm Hg. At each ADP, measurements were first determined at the moderate LAP and then at the high LAP. The experiment was completed with a repeat measurement at the high LAP at an ADP of 59 mm Hg to ensure that deterioration of the preparation had not occurred. If the results for cardiac output for the repeat measurements differed by more than 10%, the data for the experiment was discarded. After 3 minutes at each loading condition, records were made of dynamics, multiple dye dilution curves were recorded, and coronary flow and cardiac output were measured over a 1-minute period. All analog data (dye concentration, aortic flow, and LV and aortic pressure) were stored on magnetic tape for later analysis on a digital computer.

End-diastolic volumes were estimated from the dye-dilution curves. A full description of this method and the validation for measuring ventricular volumes have been published previously (Bersohn and Scheuer, 1977). Dye and aortic flow recordings were copied onto recording paper at a paper speed of 25 mm/sec. Beginning one beat after the injection of the dye bolus into the LV, dye concentration at the end of each ejection period was determined, using the aortic flow signal as a reference. These points were determined until they fell within a predetermined factor of the baseline noise. Ejection fraction (EF) was calculated from the equation 

\[ EF = 1 - \frac{K}{C} \]

where \( K = C_{n+1}/C_n \) and \( C \) is the dye concentration at beat \( n \). Ten to twelve dye dilution curves were recorded for each loading condition. For the 42 hearts that were perfused in the initial study, an average of 19.3 values for \( K \) with an average coefficient of variation of 8.18% were obtained for each determination of ejection fraction. End-diastolic volumes were estimated from the dye-dilution curves. A full description of this method and the validation for measuring ventricular volumes have been published previously (Bersohn and Scheuer, 1977). Dye dilution curves were recorded, and coronary flow and cardiac output were measured over a 1-minute period. All analog data (dye concentration, aortic flow, and LV and aortic pressure) were stored on magnetic tape for later analysis on a digital computer.

Cardiac Contractile Proteins

Hearts were stored at ~80°C in 50% glycerol containing 50 mM KCl and 10 mM KHPO4 (pH 7.0) prior to preparation of the extracts. Myosin was obtained by previously described techniques (Malhotra et al., 1981a, 1981b) and was shown by sodium dodecyl sulfate gel electrophoresis to be free of actin, tropoinin, and tropomyosin and without evidence of proteolytic degradation of myosin.

Ca++-ATPase activities were assayed at 30°C in 0.3 M KCl, 50 mM Tris-Cl (pH 7.6), 10 mM CaCl2, 5 mM ATP, and 5 mM sodium azide (Malhotra et al., 1981a, 1981b). Results are expressed as micromoles inorganic phosphate per milligram protein per minute at 30°C.

Myosin isoenzymes were analyzed by electrophoresis from crude myosin extracts (Mercadier et al., 1981) and from purified myosin on polyacrylamide gels using non-dissociating conditions at 2–3°C as reported by Hoh et al. (1977) and modified by d’Albis et al. (1979). Densitometric scans were recorded at 550 nm, and semiquantitative estimates of each isoenzyme were calculated from the height of each peak.

Serum Sex Hormone Concentrations

At the time of sacrifice of ether anesthetized animals, a 1- to 2-ml blood sample was obtained from the open chest for determination of androgen and estrogen serum concentrations. Testosterone and estradiol were assayed with commercial radioimmunoassay kits (Pentex). Sensitivity for the testosterone determination was 0.05 ng/ml and for estradiol was 10 pg/ml.

Statistical Analysis

Results for a given loading condition in perfusion studies or results for contractile proteins were submitted to a two-factor analysis of variance (Zar, 1974) utilizing sex (male/female) or gonadectomy (sham gonadectomized) as the two factors. The mean square error within groups from this analysis was then used in a Newman-Keuls multiple comparison test (Zar, 1974) to evaluate differences between hearts from male shams vs. hearts from male gonadectomized animals or hearts from female sham vs. hearts from female gonadectomized animals. In analyzing pressure-volume and force-velocity curves, we performed an analysis of covariance to test for differences in slope and elevation. Comparisons between curves for hearts from sham and gonadectomized animals were tested by a modified Newman-Keuls multiple comparison procedure (Zar, 1974). Unless otherwise stated, probabilities are reported at the 0.05 and 0.001 level.

Results

Figure 1 shows time course changes in body weight for male sham (MSH), male gonadectomized (MGX), female sham (FSH), and female gonadectomized (FGX) rats. Directionally opposite results were observed for differences between sham and gonadectomized rats of the two sexes. That is, the growth curve in MGX fell significantly below that of MSH, while the growth curve in FGX was significantly above that in FSH.

Table 1 shows results for body weight, dry left ventricular weight, dry heart weight, and the dry heart weight-to-body weight ratio at the time of...
sacrifice when animals were 18–20 weeks of age. Body weight and LV and heart weight were significantly greater in MSH than MGX, while each of these measurements were significantly less in FSH than FGX. Left ventricular weight was 18% greater in MSH than MGX and 10% less in FSH than FGX. Heart weight-to-body weight ratio was not different between the two male groups but FGX demonstrated a significantly smaller value for this ratio when compared to FSH.

Serum testosterone concentration was 3.38 ± 0.86 ng/ml (mean ± se) in MSH, but was not detectable in any of the serum samples from MGX. Serum estradiol concentration was 27 ± 4 pg/ml in FSH and was not detectable in 7 out 10 samples obtained from FGX. The detectable levels in the three FGX samples were 12, 14, and 35 pg/ml. Data from hearts from these three animals were not included in the results for cardiac dynamics in FGX. Serum testosterone concentration was 0.19 ± 0.04 ng/ml in FSH, but it was undetectable in any of the FGX samples.

Figures 2 through 7 show data obtained from the isolated heart studies. Values obtained at the end of each experiment for the repeat measurement of dynamics at high LAP/ADP = 59 mm Hg are not shown, but in all instances these values were within 10% of the values obtained earlier at the same loading condition. In Figures 2 through 7, asterisks indicate a significant difference between MSH vs. MGX or FSH vs. FGX at any combination of LAP and ADP.

Values for left atrial pressure and end-diastolic pressure (EDP) are shown in the top and middle panels of Figure 2. End-diastolic pressures of 5 mm Hg were obtained in all hearts, meeting the criteria for the moderate preload condition. LAP at this preload level was similar in MSH and MGX, but tended to be greater in FGX than FSH. For the high preload condition, end-diastolic pressures of 9–10 mm Hg were obtained in most of the hearts from MSH and FSH, but in virtually all of the hearts from MGX and FGX, mechanical alternans appeared as the EDP reached 6–7 mm Hg. Therefore, significantly lower values were observed for EDP in hearts of gonadectomized animals compared to shams at the high preload condition. The only exception was the comparison between MSH and MGX for the high preload condition at 103 mm Hg ADP where end-diastolic pressure was similar. Although EDP was lower in FGX than FSH at the high preload, LAP was the same. Measurements of end-diastolic volume normalized for dry LV weight were shown in the bottom panel of Figure 2. For both within-sex comparisons, end-diastolic volume was similar at moderate LAP but significantly less at the high LAP in hearts from gonadectomized animals.

Figure 3 shows end-diastolic pressure-volume relations at the three levels of ADP. This relation appeared to increase more steeply and shift to the left as the ADP increased. Analysis of covariance at any given ADP showed there were no statistical differences between any of the experimental groups.

Figure 4 shows values for coronary flow per g dry LV in the top panel and cardiac output per g dry LV in the bottom panel. No significant differences were observed for coronary flow although the differences in MSH and MGX were of borderline significance (P < 0.06). Cardiac output was less in hearts from

### TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>BW (g)</th>
<th>DLVW (mg)</th>
<th>DHW (mg)</th>
<th>DHW/BW (mg/g)</th>
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<tr>
<td>MSH</td>
<td>11</td>
<td>454 ± 8†</td>
<td>184 ± 2†</td>
<td>227 ± 3†</td>
<td>0.501 ± 0.010</td>
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<tr>
<td>MCX</td>
<td>10</td>
<td>387 ± 12</td>
<td>156 ± 4</td>
<td>191 ± 5</td>
<td>0.494 ± 0.006</td>
</tr>
<tr>
<td>FSH</td>
<td>10</td>
<td>275 ± 4†</td>
<td>122 ± 3*</td>
<td>150 ± 4*</td>
<td>0.545 ± 0.010†</td>
</tr>
<tr>
<td>FGX</td>
<td>8</td>
<td>344 ± 8†</td>
<td>135 ± 4</td>
<td>166 ± 6</td>
<td>0.472 ± 0.007</td>
</tr>
</tbody>
</table>

Each value represents the mean ± se. n = number of animals, BW = body weight; DLVW = dry left ventricular weight; DHW = dry heart weight; MSH = male sham; MGX = male gonadectomized; FSH = female sham; FGX = female gonadectomized.

* P < 0.05; † P < 0.001 for MSH vs. MGX or FSH vs. FGX.
gonadectomized rats at the high LAP at all levels of ADP. Furthermore, significant decreases in MGX, but not FGX, were observed at the moderate LAP at higher levels of ADP.

Figure 5 shows values for stroke work (top panel) and myocardial oxygen consumption (bottom panel), both of which are normalized for dry LV weight. As was the case for cardiac output, hearts from both male and female shams demonstrated greater values for stroke work at high LAP at all ADP. However, males differed from females, in that all of the values at the moderate LAP were decreased in MGX compared to MSH but values in FSH and FGX were similar. Differences in myocardial oxygen consumption were identical to those for stroke work. It is important to note that, for these measurements, differences between the groups at the high LAP gradually widened as the ADP was increased. Similar relationships between the groups were observed for peak left ventricular systolic pressure, although these data are not shown.

Measurements of ejection fraction (top panel) and calculated fractional shortening at the midwall (bottom panel) are shown in Figure 6. For both measurements, differences between hearts from sham and gonadectomized animals did not become apparent until the ADP was raised. Significantly lower values were observed at both moderate and high LAP in MGX at an ADP of 103 mm Hg, whereas, at 59 mm Hg, values for MSH and MGX were virtually identical. Only one instance of a significant difference in shortening was observed between FSH and
FGX, although a clear trend toward lesser muscle shortening was observed in FGX with increasing ADP.

Table 2 shows measurements of two indices of left ventricular relaxation, the maximal rate of LV pressure decline (−dP/dt) and the isovolumic relaxation period. For measurements of maximal −dP/dt, significantly slower rates were observed in hearts from male and female gonadectomized animals compared with hearts from shams at 59 mm Hg ADP, but not at 81 or 103 mm Hg ADP. In contrast, significantly longer isovolumic relaxation periods occurred in MGX compared to MSH at 81 and 103 mm Hg ADP, and were of borderline significance (P < 0.10) at 59 mm Hg ADP. A significantly longer period occurred in FGX at 59 mm Hg, with a trend for longer periods at higher levels of ADP as well.

Figure 7 shows force-velocity curves obtained by plotting mean velocity of circumferential fiber shortening (V_{cf}) vs. the mean systolic wall stress. Values shown are those obtained at moderate LAP (left panel) and high LAP (right panel) at aortic diastolic pressures of 81 and 103 mm Hg. These values of ADP were chosen since the greatest decrement in velocity was observed over this range. Also included in this figure are values obtained from hearts (n = 9) of younger male rats (YM) that were not gonadectomized (denoted by diamond symbols). This group, which had hearts 32% less in mass than the MSH group, are included to demonstrate how small male hearts perform in the isolated heart apparatus. The YM hearts were perfused at ADP of 74, 92, and 110 mm Hg, which accounts for the three points for this group in Figure 7. However, end-diastolic pressure was the same at moderate and high LAP in YM and MSH.

No differences in slope among the five groups were detected by analysis of covariance at either the moderate or high preloads. However, inspection of the data would indicate that the force-velocity relationship was qualitatively different in MSH compared to the other groups. However, small hearts from YM demonstrated slopes qualitatively similar to the FSH, FGX, and MGX groups.

A significant (P < 0.005) downward shift in the force-velocity relationship was observed in FGX compared to FSH at the moderate preload. At the high preload, this downward shift was also significant (P < 0.001) and of greater magnitude. Despite the qualitative differences in slope between MSH and MGX, covariate analysis also detected a downward shift in MGX compared to MSH at both the moderate preload (P < 0.025) and at the high preload (P < 0.01). It is interesting to note that the force-velocity relationship was virtually identical between MGX and FGX at both preloads.

Table 3 shows results obtained at the high preload condition from the ancillary study where end-diastolic pressure was equated in FSH and FGX. Mean heart rate was 327 beats/min in FSH and 314 beats/min in FGX; these differences were not significant. End-diastolic pressure was relatively well-matched between FSH and FGX at any given ADP. Similarly, end-diastolic volume was not different. Decreased pump performance in FGX was evidenced by declines in stroke volume (per g LV) and peak LV pressure. Furthermore, a decreased contractile state in FGX was suggested by decreases in ejection fraction and maximum V_{cf}. These differences occurred at virtually all levels of ADP.

Table 4 reports values for Ca^{++}-stimulated myosin ATPase, K^{+}-EDTA myosin ATPase, and the distribution of myosin isoenzymes in the experimental groups. Ca^{++}-myosin ATPase was reduced (P < 0.001) by 34% in MGX compared to MSH and was reduced (P < 0.001) by 19% in FGX compared to FSH. K^{+}-EDTA ATPase was not different for the same group comparisons. Figure 8 shows representative gel patterns and representative densitometric
Discussion

In the present study, body growth was suppressed in gonadectomized male animals relative to male shams, but was elevated in gonadectomized female animals relative to female shams. These effects of prepuberal gonadectomy have been reported previously (Koos Slob and Van Der Werff Ten Bosch, 1975; Grunt, 1964). In addition, our current results indicate that gonadectomy also affects cardiac growth in a similar manner in male and female rats. Koenig et al. (1982) have recently shown a similar effect of gonadectomy on cardiac mass in male mice and rats. These workers also found increases in total RNA and protein in hearts of testosterone-treated female mice, suggesting that this hormone exerts an anabolic effect on the ventricular myocardium.

One of the most prominent features of hearts from gonadectomized rats was the inability to achieve end-diastolic pressures equivalent to those in hearts from sham-operated rats at high left atrial pressures. These results suggest a defect in left ventricular filling in hearts from gonadectomized rats. Further increases in LAP in MGX and FGX were associated with the onset of mechanical alternans and a decline in ventricular performance. The lower end-diastolic pressures in MGX and FGX were not due to differences in heart sizes, since MSH had the largest heart mass and FSH had the smallest heart mass, and both of these groups demonstrated normal end-diastolic pressures at the high LAP. Neither was it likely that the lower end-diastolic pressures in MGX and FGX represented an increase in left ventricular compliance, since the end-diastolic pressure-volume relationship was not shifted to the right in hearts from gonadectomized animals. Furthermore, in the follow-up study, when end-diastolic pressure was equated between FSH and FGX at the high LAP, end-diastolic volume in the two groups was similar.

Left ventricular filling can be affected by alterations in left-ventricular relaxation (Yellin et al., 1980). At the ADP of 59 mm Hg, we observed a significantly slower maximum rate of LV pressure.
decline in both MGX and FGX, but these differences were not detected at higher aortic diastolic pressures. We suspect this measurement to have had a higher frequency content with increases in afterload, and it was likely that the frequency response of the LV catheter-manometer was not sufficient to measure this accurately. However, significantly longer isovolumic relaxation periods, another index of ventricular relaxation, were observed in MGX at the higher aortic diastolic pressures. A relaxation defect in hearts from gonadectomized animals could account for the low end-diastolic pressure at high LAP observed in these hearts. Yellin et al. (1980) have shown in dogs that slower relaxation diminishes the atrial-ventricular pressure gradient in early diastole, resulting in a slower rate of ventricular filling, and a lower ventricular diastolic pressure.

Differences in contractile performance between MSH and MGX were evidenced by significant decreases in MGX for cardiac output, stroke work, peak LV pressure, ejection fraction, and fractional shortening. At high levels of LAP, these differences were associated with lower end-diastolic pressures and volumes in MGX. At the highest level of ADP, end-diastolic pressures—but not end-diastolic volume—were similar at high LAP in MSH and MGX, and the largest differences in left ventricular function were observed at this loading condition. Furthermore, at aortic diastolic pressure levels of 81 and 103 mm Hg, differences in function were also observed between MSH and MGX at moderate LAP where end-diastolic pressure and volume were similar. The finding of decreased muscle shortening in MGX under conditions of similar end-diastolic pressure and volume are suggestive of a decreased inotropic state in MGX. Furthermore, the largest differences in function between MSH and MGX occurred at the highest ADP, suggesting an altered response to afterload in MGX.

In the initial study, assessment of differences in cardiac function in FSH and FGX was more difficult than in the male groups, since virtually all of the differences between the female groups occurred at the high LAP where both end-diastolic pressure and volume were significantly lower in FGX. Therefore, it may be argued that FSH and FGX were not compared at comparable preloads, and this may account artificially for the observed decreases in function. However, when force-velocity plots obtained from measurements of mean \( V_{ef} \) and mean systolic wall stress were analyzed, a significant downward shift in this relationship was observed in FGX compared with FSH at both moderate and high preloads. The mean wall stress-mean velocity rela-

![Figure 5. Values for stroke work (top panel) and myocardial oxygen consumption (bottom panel) for hearts from male and female sham and gonadectomized animals. Both measurements are normalized for dry left ventricular weight. All other notations are the same as in Figure 2.](image-url)
Fratantion can be used as an index of inotropic state in the intact heart (Sasayama et al., 1977). Therefore, a decrease in contractility was suggested in FGX at the moderate preload where end-diastolic pressure and volume were similar in FSH and FGX. In a second study, when hearts from FGX were studied at slightly greater end-diastolic pressures which were equal to those in FSH, decreases in stroke volume, ejection fraction, and peak $V_d$ were observed, which confirmed a decreased contractile state in FGX.

Gonadectomy in both male and female rats led to a decrease in cardiac Ca++-myosin ATPase activity which was associated with a reduction in the content of the $V_1$ isoenzyme and an increase in the content of the $V_3$ isoenzyme. Recently, Schwartz et al. (1981)

### Table 2

**Indices of Left Ventricular Relaxation**

<table>
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<tr>
<th></th>
<th>ADP - 59 mm Hg</th>
<th></th>
<th>ADP - 81 mm Hg</th>
<th></th>
<th>ADP - 103 mm Hg</th>
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<tr>
<td></td>
<td>Mod LAP</td>
<td>High LAP</td>
<td>Mod LAP</td>
<td>High LAP</td>
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<tr>
<td>Maximal $-dP/dt$ (mm Hg/sec)</td>
<td>-2920 ± 71*</td>
<td>-2860 ± 52*</td>
<td>-3720 ± 76</td>
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<tr>
<td>MSH</td>
<td>-2390 ± 57</td>
<td>-2310 ± 79</td>
<td>-3310 ± 129</td>
<td>-3290 ± 122</td>
<td>-3970 ± 181</td>
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<tr>
<td>MGX</td>
<td>-2970 ± 192*</td>
<td>-2730 ± 182</td>
<td>-3450 ± 138</td>
<td>-3570 ± 155</td>
<td>-4150 ± 245</td>
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<tr>
<td>FGX</td>
<td>-2390 ± 124</td>
<td>-2380 ± 70</td>
<td>-3490 ± 150</td>
<td>-3400 ± 145</td>
<td>-3980 ± 220</td>
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<tr>
<td>Isovolumic relaxation period (msec)</td>
<td>10.7 ± 0.5*</td>
<td>14.3 ± 0.5</td>
<td>20.7 ± 0.5*</td>
<td>20.4 ± 0.7*</td>
<td>29.2 ± 1.0</td>
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<tr>
<td>MSH</td>
<td>17.5 ± 0.8</td>
<td>17.7 ± 0.8</td>
<td>27.2 ± 1.7</td>
<td>28.3 ± 1.9</td>
<td>36.4 ± 2.3</td>
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<tr>
<td>MGX</td>
<td>14.6 ± 0.9</td>
<td>13.2 ± 1.3*</td>
<td>26.1 ± 1.6</td>
<td>24.4 ± 1.7</td>
<td>36.2 ± 1.9</td>
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<tr>
<td>FGX</td>
<td>18.7 ± 1.9</td>
<td>18.3 ± 2.0</td>
<td>30.1 ± 2.7</td>
<td>29.5 ± 2.5</td>
<td>41.0 ± 2.8</td>
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</table>

Results are the mean ± SEM for 10-11 hearts. LAP = left atrial pressure; ADP = aortic diastolic pressure. Other notations are the same as in Table 1.
have associated an increase in V3 content with a decrease in the maximum unloaded shortening velocity in papillary muscles from rats with chronic cardiac overload. Thus the abnormalities in myosin enzymes in hearts from MGX and FGX may underly the observed differences in contractile performance of these hearts.

The observation of significant decreases in pump

TABLE 3
Dynamics in Female Hearts with Equal End-Diastolic Pressure

<table>
<thead>
<tr>
<th></th>
<th>ADP - 59 mm Hg</th>
<th>ADP - 81 mm Hg</th>
<th>ADP - 103 mm Hg</th>
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</thead>
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<tr>
<td>End-diastolic pressure (mm Hg)</td>
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<td></td>
</tr>
<tr>
<td>FSH</td>
<td>8.5 ± 1.0</td>
<td>9.2 ± 0.7</td>
<td>9.5 ± 0.9</td>
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<td>FGX</td>
<td>7.1 ± 0.6</td>
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<td>8.5 ± 0.7</td>
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<td>End-diastolic volume (ml/g)</td>
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<tr>
<td>FSH</td>
<td>2.94 ± 0.07</td>
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<td>2.88 ± 0.13</td>
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<td>Stroke volume (ml/g)</td>
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</tr>
<tr>
<td>FSH</td>
<td>1.93 ± 0.04*</td>
<td>1.62 ± 0.04*</td>
<td>1.30 ± 0.02†</td>
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<tr>
<td>FGX</td>
<td>1.69 ± 0.06</td>
<td>1.41 ± 0.06</td>
<td>1.08 ± 0.05</td>
</tr>
<tr>
<td>Peak LV pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSH</td>
<td>150 ± 2</td>
<td>139 ± 3*</td>
<td>155 ± 3*</td>
</tr>
<tr>
<td>FGX</td>
<td>144 ± 2</td>
<td>132 ± 2</td>
<td>146 ± 2</td>
</tr>
<tr>
<td>Ejection fraction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSH</td>
<td>0.66 ± 0.01*</td>
<td>0.62 ± 0.01*</td>
<td>0.56 ± 0.01†</td>
</tr>
<tr>
<td>FGX</td>
<td>0.59 ± 0.02</td>
<td>0.54 ± 0.01</td>
<td>0.48 ± 0.01</td>
</tr>
<tr>
<td>Peak Vcf (circ/sec)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FSH</td>
<td>4.54 ± 0.05†</td>
<td>4.27 ± 0.07†</td>
<td>3.87 ± 0.05†</td>
</tr>
<tr>
<td>FGX</td>
<td>3.78 ± 0.14</td>
<td>3.53 ± 0.13</td>
<td>3.06 ± 0.12</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SEM of seven hearts. All results were obtained at the high preload condition (defined in text).

* P < 0.05; † P < 0.001 for FSH vs. FGX by unpaired t-analysis. Other notations are the same as in Table 1.
TABLE 4
Cardiac Contractile Proteins

<table>
<thead>
<tr>
<th></th>
<th>MSH (4)</th>
<th>MGX (4)</th>
<th>FSH (4)</th>
<th>FGX (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca(^{++})-myosin ATPase ((\mu)mol Pi/mg per min)</td>
<td>0.95 ± 0.04(\dagger)</td>
<td>0.63 ± 0.02</td>
<td>1.10 ± 0.01(\dagger)</td>
<td>0.89 ± 0.03</td>
</tr>
<tr>
<td>K(^{+})-EDTA myosin ATPase ((\mu)mol Pi/mg per min)</td>
<td>1.10 ± 0.02</td>
<td>1.18 ± 0.06</td>
<td>1.06 ± 0.04</td>
<td>1.00 ± 0.03</td>
</tr>
<tr>
<td>Myosin isoenzymes (% content)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V(_1)</td>
<td>66 ± 5(\dagger)</td>
<td>25 ± 2</td>
<td>76 ± 3(\ast)</td>
<td>54 ± 9</td>
</tr>
<tr>
<td>V(_2)</td>
<td>22 ± 3(\ast)</td>
<td>35 ± 1</td>
<td>19 ± 3(\ast)</td>
<td>31 ± 5</td>
</tr>
<tr>
<td>V(_3)</td>
<td>12 ± 2(\dagger)</td>
<td>40 ± 2</td>
<td>5 ± 1(\ast)</td>
<td>15 ± 5</td>
</tr>
</tbody>
</table>

Results are the mean ± SEM for the number of hearts shown in parentheses. Other notations, including symboled footnotes, are the same as in Table 1.

and muscle function at moderate LAP in MGX, but not in FGX, suggests that the cardiac effects of gonadectomy were greater in male rats. However, in assessing these relative effects, one must consider the directionally opposite differences in heart size between male and female gonadectomized and sham animals. Clues to the effects of heart size on performance of hearts in the working heart apparatus were apparent in MSH, which had the largest heart size of all the groups. In contrast to the small hearts of the other groups, only small decrements in mean V\(_{cf}\) were observed in MSH as ADP was increased. Hearts from male shams also demonstrated a qualitatively different slope in the mean force-velocity relationship. That this altered response in MSH was due to their large heart size is supported by the finding of a qualitatively similar slope to FSH, FGX, and MGX in smaller hearts from younger male control rats. We have recently completed a study (Schaible et al., 1982) correlating differences in heart mass to differences in functional measurements. This study has shown that virtually all dynamic measurements that are normalized by a measure of heart size decrease as heart mass increases. For example, a 10% decrease in cardiac output per g LV would be predicted in hearts of the same mass as MSH compared to hearts of the same mass as MGX. Conversely, an increase of 4% would be predicted in hearts equal in mass to FSH compared to hearts equal in mass to FGX. It is difficult to attribute differences in heart mass to quantitative differences in functional measurements, but these effects of heart mass would tend to diminish differences between MSH and MGX and slightly enhance differences between FSH and FGX. These directionally opposite effects of heart size on the comparison of cardiac function in MGX and FGX support the conclusion that the cardiac effects of gonadectomy were greater in male rats. Furthermore, a larger relative reduction in myosin ATPase activity was observed in MGX (34%) compared to that observed in FGX (19%).

Recent studies demonstrating specific sex hormone receptors in heart muscle would suggest that the heart is a target organ for gonadal hormones. Androgen receptors have been demonstrated in male and female rat and baboon ventricular and atrial myocardium (Krieg et al., 1978, 1980; McGill and Sheridan, 1981; McGill et al., 1980), as well as in coronary vessels of baboons (McGill and Sheridan, 1981) and dogs (Horwitz and Horwitz, 1981). However, estrogen receptors have been demonstrated only in rat atrial myocardium and not in ventricular myocardium (Stumpf et al., 1977). Evidence for decreased left ventricular contractility in both MGX and FGX was obtained in the present study, but this finding in FGX is not consistent with the reported absence of estrogen receptors in rat ventricular tissue. Instances where sex hormone effects are not mediated through receptor mechanisms have been reported (Bardin and Catteral, 1980) so it
is possible that the effects of estrogens and androgens on the ventricular myocardium may be through different mechanisms. Autoradiographic studies (McGill et al., 1980; McGill and Sheridan, 1981) showing nuclear uptake of androgens in ventricular myocardial fibers of baboons and in vitro studies (Krieg et al., 1980) showing binding of cytoplasmic androgen receptors to rat myocardial nuclei suggest that the effects of androgens on myosin enzymes may be exerted at the transcriptional level.

In summary, the present findings of abnormal cardiac function and biochemistry resulting from gonadectomy, along with the recent demonstration of specific sex hormone receptors in heart muscle, suggest that the sex hormones may be involved in regulation of cardiac function. Whether the sex hormones affect the myocardium directly or by secondary mechanisms remains to be investigated. Also, our results apply to hearts from rats that were gonadectomized before puberty and then studied as adults. Therefore, we cannot determine whether the effect is due to the influence of sex hormones on cardiac development or whether the effects of the sex hormones are exerted continuously in adult animals. This question requires further investigation.

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References

Altura BM (1975) Sex and estrogens and responsiveness of terminal arterioles to neurohypophysial hormones and catecholamines. J Pharmacol Exp Ther 193: 403–412


INDEX TERMS: Gonadectomy • Sex hormones • Left ventricular function • Left ventricular filling • Force-velocity relationships • Cardiac contractile proteins
The effects of gonadectomy on left ventricular function and cardiac contractile proteins in male and female rats.

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