It has become apparent that the use of general anesthesia for some types of hemodynamic investigations introduces changes which may limit application of the results to normal physiology. The importance of this point has been emphasized by Rushmer and his colleagues and studies of unanesthetized animals have been published by several authors in recent years. Certainly most cardiovascular experiments in animals are more difficult without general anesthesia and some are impossible. However, those requiring only catheterization of the vascular system can be accomplished with local anesthesia, and in the untrained animal are facilitated by the use of a tranquilizing drug.

This report describes left ventricular volume measurements by thermodilution in dogs that had received the tranquilizer perphenazine* plus local anesthesia, but no general anesthesia. In addition, the effects of intravenous infusions of norepinephrine and methoxamine were studied.

Methods

Fifteen dogs were given perphenazine intravenously in a dose range from 0.55 to 1.10 mg/kg. Each animal was then placed on his side, usually without restraints. One member of the experimental team attended continuously to the animal's comfort, providing a reassuring monologue and frequent head patting. Lidocaine was infiltrated below the inguinal ligament bilaterally and both superficial femoral arteries and one femoral vein were exposed. With adequate local anesthesia, this was accomplished during elevation of the uppermost hindquarter, without necessity for the supine position. A 7F catheter with a closed end and multiple side holes was introduced into a femoral artery and advanced into the left ventricle, during continuous monitoring of pressure. A 6F catheter was also advanced into the left ventricle from the other femoral artery and then withdrawn 1 cm beyond the point at which left ventricular pressure changed to aortic pressure. A small nylon catheter with a rapidly responding bead thermistor at its tip was put through the 6F catheter so that its tip was in the aortic root, extending just beyond the end of the 6F catheter. The relative lengths of these catheters were predetermined. Another 7F catheter was put into a femoral vein and advanced into the inferior vena cava.

Left ventricular pressure was measured with a Statham P23Db strain gauge and a direct-writing Gilson oscillograph. The zero reference level was at the mid-thorax, with the animal on his side. Cardiac output was determined by dye dilution, with injection of 2.5 mg of indocyanine green into the inferior vena cava and sampling from the left ventricle. Blood was withdrawn by a mechanical pump through a Waters X300 densitometer and reinfused after inscription of the curve. Curves were calibrated by drawing arterial blood through the densitometer without dye added and with three known concentrations of dye.

Aortic thermodilution curves were obtained by rapid injections of 2 to 5 ml of cooled normal saline into the left ventricle. The temperature changes thus produced were measured by the aortic thermistor. The basis of the method, its reproducibility and the equipment employed have been described previously. In the present studies stroke volume (SV) was measured by dividing the dye dilution cardiac output by the heart rate, and the thermodilution curves were used to measure the ratio of left ventricular end-systolic volume (ESV) to end-diastolic volume (EDV). From these two observations, ESV and EDV were calculated.

The procedure for experiments was as follows: Left ventricular pressure was recorded, and then three to six thermodilution curves obtained. The cardiac output determination followed immedi
ately. This sequence usually required about three minutes. Such a series of measurements was made twice in the resting, control state. In four dogs, the response to a vasodilator was tested by intravenous administration of 25 mg of aminephylline each two minutes to a total dose of 75 to 200 mg.

In nine animals intravenous infusions of norepinephrine and methoxamine were given. Thirty minutes after cessation of the first drug, a single set of control measurements was repeated, followed by infusion of the other drug. Norepinephrine was the first agent given in six animals and methoxamine was first in three. In four of the dogs, an attempt was made to administer "equipressor" amounts of these agents, using two doses of each drug in each animal. In these experiments the norepinephrine dose was from 1.4 to 5.9 μg/kg/min, and the methoxamine dose was from 45 to 110 μg/kg/min. This approach was abandoned when it became evident that a significant increase in left ventricular systolic pressure was difficult to produce in some animals despite large amounts of drug. Arbitrary doses were then adopted in the remaining five animals. For norepinephrine these were 0.25 and 0.50 μg/kg/min, and for methoxamine 50 and 100 μg/kg/min. In the final calculations the findings at various dose levels were averaged. Ventricular volumes, cardiac output, heart rate and left ventricular systolic pressure during drug administration were compared to the control measurements obtained immediately before. Differences between control and drug periods were evaluated by the

\[ t = \frac{\sum D}{\sqrt{N(\sum D^2) - (\sum D)^2}} N - 1 \]

where:

- \( D \) = algebraic difference between control and test period in a given animal.
- \( N \) = number of animals.

Probability values were obtained from a t-table and values less than 0.05 were considered significant.

We wished to study the effects of norepinephrine and methoxamine upon the relationship between resting ventricular muscle fiber length and the subsequent contractile force developed by the myocardium during systole, in the intact animal. In order to approach this problem certain assumptions were necessary. EDV was employed as a measure of resting fiber length. The ventricle was assumed to act as a thin-walled sphere obeying the Laplace law. Since the instantaneous change in left ventricular volume with systole was not available, an estimate of mid-systolic ventricular wall tension, or force, was obtained from these formulas:

1) Mid-systolic ventricular volume (ml)

\[ = EDV - \frac{SV}{2} \]

2) Mid-systolic ventricular wall force (kg)

\[ = \frac{PrR^2}{1000} \]

where \( P \) = peak left ventricular systolic pressure (g/cm²) and \( R \) = internal radius of a spherical ventricle at the mid-systolic volume.

These equations required another assumption, which was that peak pressure in the ventricle was present at the time that one-half of the stroke volume had been ejected. This appeared justified after inspection of published recordings from flowmeters around the aortic root and simultaneous left ventricular pressure measurements. The time at which half of the stroke volume had been ejected was associated with peak, or very close to peak, left ventricular pressure.

EDV was plotted against mid-systolic force for observations during norepinephrine infusion and the control values immediately preceding its administration. Similarly the distribution of EDV and force values for methoxamine was compared with the corresponding controls. Regression lines and correlation coefficients were calculated for both sets of control and test data. Using the regression slope for the control observations, each control force value was adjusted to the average control EDV. This provided a distribution of adjusted force measurements at the average EDV. The test force values (norepinephrine or methoxamine) were similarly adjusted from their regression equation to this same EDV and provided another distribution of force values at the average control EDV. Since variations in EDV were "corrected" by this maneuver, the control and test distributions could be compared by the \( t \) test. The foregoing calculations were performed by a computer.*

**Results**

The left ventricle could be catheterized with relative ease without fluoroscopy and without distress for the animal. The dogs appeared relaxed, occasionally slept, but were always responsive. At the conclusion of the experiments they walked without difficulty.

When the duplicate measurements in the

*Scientific Data Systems 290.

Circulation Research, Vol. XVIII, April 1966
first control period were compared with each other, no statistical differences were found. In the 15 dogs, the mean values for the duplicate measurements were: heart rate 101 and 105; cardiac output, 2.51 and 2.56 liters/min; ESV/EDV 61 and 59%. Each set of duplicate determinations and the quantities derived from them were averaged and the results are presented in Table 1. The difference of a given cardiac output determination from the average of each pair, without regard to sign, was 6.4% ± 5.5% (mean ± SD).

The control observations were usually obtained during an apparent steady state. The responsiveness of the cardiovascular system was demonstrated, however, by one animal who slept during the first set of measurements and then raised his head and looked about during the second set. The heart rate rose from 75 to 139, the cardiac output from 1.67 to 2.63 liters/min, and left ventricular systolic pressure from 115 to 143. EDV and ESV decreased about 20 and 25%, respectively.

The heart rate and left ventricular systolic pressure were considerably lower than we observed in dogs anesthetized with chloralose and urethane, and cardiac output and stroke volume on a weight basis were higher.2-4 Sinus arrhythmia was always present. The left ventricular volume measurements showed that the ventricle emptied about 40% of its EDV as the stroke volume (Fig. 1). The ventricular EDV averaged 4.7 ml/kg which is more than twice the value we observed previously during anesthesia.

The ability of the preparation to respond to a vasodilator was tested with intravenous aminophylline in four animals; tachycardia and increased cardiac output were produced in each. The average control heart rate of 95 rose to 148 and cardiac output changed from 2.41 liters/min to 3.76. EDV and ESV decreased slightly.

In nine dogs given norepinephrine a moderate increase in left ventricular systolic pressure was found with some cardiac slowing and an increase in SV (Table 2). The latter increase was proportionately greater than the decrease in heart rate, and thus cardiac output was higher. EDV increased but no statistically significant change was found for ESV/EDV or for ESV. Since EDV and SV were shown to increase, it must be that either

**Table 1**

<table>
<thead>
<tr>
<th>Weight (kg)</th>
<th>Heart rate (beats/min)</th>
<th>Cardiac output (liters/min)</th>
<th>Stroke volume (ml)</th>
<th>ESV/EDV* X 100</th>
<th>EDV (ml)</th>
<th>ESV (ml)</th>
<th>LV pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.4</td>
<td>59</td>
<td>1.61</td>
<td>27</td>
<td>54</td>
<td>59</td>
<td>140/4</td>
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<tr>
<td>2</td>
<td>14.1</td>
<td>76</td>
<td>1.57</td>
<td>28</td>
<td>66</td>
<td>74</td>
<td>128/12</td>
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<tr>
<td>3</td>
<td>11.4</td>
<td>107</td>
<td>2.15</td>
<td>20</td>
<td>56</td>
<td>46</td>
<td>159/7</td>
</tr>
<tr>
<td>4</td>
<td>13.2</td>
<td>138</td>
<td>3.88</td>
<td>28</td>
<td>53</td>
<td>60</td>
<td>139/0</td>
</tr>
<tr>
<td>5</td>
<td>10.9</td>
<td>116</td>
<td>2.01</td>
<td>17</td>
<td>70</td>
<td>57</td>
<td>136/12</td>
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<tr>
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<td>12.3</td>
<td>92</td>
<td>2.35</td>
<td>26</td>
<td>54</td>
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<tr>
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<td>11.1</td>
<td>80</td>
<td>1.16</td>
<td>15</td>
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<td>42</td>
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<tr>
<td>8</td>
<td>11.8</td>
<td>165</td>
<td>2.51</td>
<td>15</td>
<td>51</td>
<td>31</td>
<td>143/3</td>
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<tr>
<td>9</td>
<td>9.0</td>
<td>138</td>
<td>2.26</td>
<td>16</td>
<td>58</td>
<td>38</td>
<td>142/11</td>
</tr>
<tr>
<td>10</td>
<td>12.7</td>
<td>94</td>
<td>2.29</td>
<td>24</td>
<td>60</td>
<td>60</td>
<td>130/8</td>
</tr>
<tr>
<td>11</td>
<td>15.0</td>
<td>140</td>
<td>3.72</td>
<td>27</td>
<td>67</td>
<td>82</td>
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<tr>
<td>12</td>
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<td>77</td>
<td>1.42</td>
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<tr>
<td>13</td>
<td>13.5</td>
<td>81</td>
<td>2.74</td>
<td>34</td>
<td>60</td>
<td>85</td>
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<td>17.7</td>
<td>89</td>
<td>4.10</td>
<td>46</td>
<td>51</td>
<td>94</td>
<td>143/13</td>
</tr>
<tr>
<td>15</td>
<td>24.5</td>
<td>96</td>
<td>3.88</td>
<td>40</td>
<td>59</td>
<td>98</td>
<td>154/14</td>
</tr>
</tbody>
</table>

Mean ± SD: 13.6 ± 30 2.53 ± 0.95 25.2 ± 9.2 59.9 ± 7.2 63.7 ± 20.2 37.8 ± 12.5

*EDV: end-diastolic left ventricular volume, ESV; end-systolic left ventricular volume.

†Left ventricular diastolic pressure was measured at end-diastole.
FIGURE 1
Representative thermodilution curves during control periods from three animals. A decrease in aortic blood temperature is an upward deflection, and was always less than a peak change of 1.0°C. Left ventricular pressure was displayed on the oscilloscope only as a time reference for the thermal curves; measurements of pressure were made from recordings with a separate oscillograph.

ESV or the ratio ESV/EDV changed. The average values showed ESV had increased and ESV/EDV had decreased slightly. Thus an increase in SV was associated with a larger EDV, with suggestive evidence of more complete emptying of the larger ventricle.

During methoxamine administration the major change was a decrease of cardiac output of 25%. Left ventricular EDV, ESV and ESV/EDV were not altered significantly (table 2).

The correlations and regression equations for the plots of mid-systolic force vs. EDV are shown in table 3. The slope of the norepinephrine distribution was steeper than the control slope. As described earlier, the force values were adjusted to a fixed EDV to facilitate statistical comparison. When measurements during norepinephrine infusion were compared with their preceding control data, higher force values were found for norepinephrine. This indicated that contractions which began from a given EDV produced a greater mid-systolic force during norepinephrine administration than before it (P = <0.02). There was no difference between measurements before and during methoxamine administration (P = >0.05).

Discussion
EVALUATION OF THE PREPARATION AND COMPARISON WITH ANESTHETIZED ANIMALS

The control findings in our animals differ significantly from those in anesthetized dogs. The consistent presence of sinus arrhythmia, the lower heart rates, and lower left ventricular systolic pressure, as well as the higher cardiac outputs, were differences which were striking to us when compared to previous experience with various anesthetic agents.2-4,11

The only pharmacological agent used during our control measurements was perphenazine, the sedative effects of which made the studies possible in untrained animals. The relatively slow heart, sinus arrhythmia, the responsiveness of the animals, as well as the values for SV and cardiac output suggest that the dogs are reasonably comparable to those without anesthesia. The response to intravenous aminophylline was appropriate and showed considerable increases of heart rate and cardiac output. This response, as well as the difficulty in producing sustained left ventricular systolic hypertension with vasopressors in some animals, suggested the presence of a well functioning autonomic system and a reactive cardiovascular system. We believe
### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Heart rate</th>
<th>Cardiac output</th>
<th>Stroke volume</th>
<th>ESV/EDV (\times 100^*)</th>
<th>EDV</th>
<th>ESV</th>
<th>Left ventricular systolic pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>103 ± 31</td>
<td>1.99 ± 0.54</td>
<td>20.0 ± 0.1</td>
<td>62.9 ± 7.8</td>
<td>54.6</td>
<td>34.4</td>
<td>150 ± 21</td>
</tr>
<tr>
<td>Norpinephrine</td>
<td>90 ± 20(^c)</td>
<td>2.44 ± 0.66(^c)</td>
<td>27.9 ± 7.3(^a)</td>
<td>57.8 ± 9.8(^a)</td>
<td>68.0</td>
<td>40.5</td>
<td>179 ± 28(^a)</td>
</tr>
<tr>
<td>Control</td>
<td>98 ± 20(^d)</td>
<td>2.15 ± 0.72</td>
<td>21.4 ± 4.0</td>
<td>61.5 ± 10.4</td>
<td>58.4</td>
<td>37.0</td>
<td>153 ± 29</td>
</tr>
<tr>
<td>Methoxamine</td>
<td>91 ± 31(^e)</td>
<td>1.62 ± 0.57(^d)</td>
<td>18.0 ± 3.9(^e)</td>
<td>66.0 ± 6.4(^e)</td>
<td>54.8</td>
<td>36.9</td>
<td>164 ± 27(^d)</td>
</tr>
</tbody>
</table>

Means ± 1 standard deviation are listed.

*EDV: end-diastolic left ventricular volume, ESV: end-systolic left ventricular volume.

a: \(P < 0.001\)
b: \(P < 0.010\)
c: \(P < 0.025\)
d: \(P < 0.050\)
e: ns; not significant

These findings suggest that perphenazine did not exert a significant depressant effect upon the circulation. The step-to-step downslopes of the thermodilution curves were more variable than in other studies during anesthesia. This is in part related to the higher ESV/EDV, which provided varying periods of ventricular dilatation and therefore variable ventricular diastolic filling and therefore end-diastolic volume. The individual downslopes were averaged to obtain an average value for ESV/EDV. The ESV/EDV indicates the proportion of the ESV remaining at the end of diastole and thus reflects the degree of ventricular emptying. In previous work in the dog this ratio was found to average 79% during pentobarbital anesthesia, 2 and 66% and 70% in two investigations with chloralose-urethane anesthesia, 3,4 compared to 60% in the unanesthetized animals reported here. The individual downslopes were averaged to obtain an average value for ESV/EDV. This ratio was found to average 79% during pentobarbital anesthesia, 2 and 66% and 70% in two investigations with chloralose-urethane anesthesia, 3,4 compared to 60% in the unanesthetized animals reported here. The individual downslopes were averaged to obtain an average value for ESV/EDV. These findings are consistent with observations by Van Citters et al. before and during anesthesia in the dog. They observed tachycardia, a decreased SV, and smaller diastolic left ventricular diameter with anesthesia. It is possible that the depressant effects of general anesthesia on cardiac performance are, in part related to the lower ESV/EDV, because a faster heart rate during anesthesia, because a faster heart rate occurs during anesthesia, because a faster heart rate produces a decrease in SV, which depressant effect upon the circulation.
TABLE 3

End-Diastolic Volume and Mid-Systolic Force Distributions in Nine Dogs

<table>
<thead>
<tr>
<th></th>
<th>Regression equation</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-norepinephrine control</td>
<td>$F = 0.039 \text{EDV} + 0.90$</td>
<td>0.90</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>$F = 0.046 \text{EDV} + 1.08$</td>
<td>0.81</td>
</tr>
<tr>
<td>Pre-methoxamine control</td>
<td>$F = 0.043 \text{EDV} + 0.59$</td>
<td>0.73</td>
</tr>
<tr>
<td>Methoxamine</td>
<td>$F = 0.041 \text{EDV} + 1.15$</td>
<td>0.75</td>
</tr>
</tbody>
</table>

*F: mid-systolic force, in kg.
†EDV: end-diastolic volume, in ml.

decrease of left ventricular size, and less complete ventricular emptying.

EFFECTS OF NOREPINEPHRINE AND METHOXAMINE

During norepinephrine infusion the results indicated a larger ventricle, associated with a slower heart, and a tendency toward more complete ventricular emptying. The larger EDV could have been a result of both the higher impedance to outflow and the slower heart rate. With an increased EDV the larger SV conceivably could have resulted from more forceful contraction due only to longer diastolic muscle fiber length. However, the known positive inotropic effect of norepinephrine was demonstrated by the mid-systolic force data which showed greater contractile force from a given EDV during norepinephrine infusion.

Though cardiac output was reduced significantly by methoxamine, no statistically significant changes were found for SV or other ventricular volumes. Similarly, no alteration in the relationships between EDV and mid-systolic force was present, consistent with a lack of significant inotropic influence on the heart by methoxamine, a conclusion reached by previous workers.

Other studies of changes of left ventricular volume or dimensions have been performed with these and similar agents. In anesthetized dogs, Eckstein and Wendling showed that norepinephrine improved ventricular emptying which had been depressed during hexamethonium administration. However, the ESV/EDV ratios with norepinephrine were not lower than the preceding control measurements, as in our animals. In man, Harrison et al. demonstrated an increase in diastolic and systolic left ventricular dimensions with methoxamine infusion. Though EDV did not increase in our studies of dogs, we believe that the conclusions are similar, indicating lack of positive inotropic activity by methoxamine.

It must be acknowledged that the force calculations employed here are intended only as indices of ventricular performance, as described earlier. It is also apparent that the velocity of muscle fiber shortening, an important characteristic of contraction, is not described by our studies. However, the results do combine measurements of both the size of the ventricle and its systolic pressure, and permit separation of effects due to changes in fiber length from other inotropic influences.

Summary

Left ventricular volumes were estimated by thermodilution in unanesthetized dogs given the tranquilizer, perphenazine. During systole, the left ventricle emptied an average of 40% of its end-diastolic volume. This is a significantly greater proportion than observed previously during general anesthesia with several different agents.

Left ventricular mid-systolic wall force was calculated. Higher force values at a given end-diastolic volume occurred during intravenous infusion of norepinephrine. Methoxamine failed to change these relationships.

Acknowledgment

We express appreciation for the help of Dr. Lyle Calvin, Professor of Statistics, Oregon State University, and Dr. Lee Lusted, Department of Biomathematics, Oregon Regional Primate Research Center, who designed and performed the statistical analyses.

We are indebted to Dr. James Metcalfe, Depart-
ment of Medicine, in whose laboratory these studies were performed.

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Left Ventricular Volume Studies in Tranquilized Dogs with Local Anesthesia
J. David Bristow, Cyrus Farrehi and Kent Ueland

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