Effects of Cyclopropane and of Hypoxia on Transmembrane Potentials of Atrial, Ventricular and Purkinje Fibers

By Larry D. Davis, Ph.D., John V. Temte, B.Sc., Phyllis R. Helmer, B.Sc., and Quillian R. Murphy, M.D., Ph.D.

The action of cyclopropane in sensitizing the myocardium to catecholamines has been the subject of numerous investigations. Little information is available on the effects of cyclopropane alone on cellular processes in cardiac tissue. Recently, however, using electrophysiological recording techniques, Smith et al. found that the ventricular strength-interval curve shifted to the right in dogs anesthetized with cyclopropane. This was interpreted as indicating a decrease in ventricular excitability. Levy et al. studied the effects of cyclopropane on transmembrane potentials of rabbit atrium and ventricular fibers of dogs anesthetized with cyclopropane. The action potential was shortened in duration in the former tissue while no changes could be detected in in situ ventricular muscle fibers. Recent studies of Moore et al. suggest that the ventricular arrhythmias elicited by catecholamines in dogs treated with cyclopropane arise in the ventricular conducting system. It is possible that cyclopropane exerts on cardiac tissues some direct effect which is in part responsible for the development of arrhythmias. The present paper reports the effects of cyclopropane and of hypoxia on transmembrane potentials of single Purkinje, ventricular, and atrial muscle fibers.

Methods

Hearts were excised from dogs anesthetized with cyclopropane (33% in oxygen) or sodium pentobarbital (30 mg/kg administered intravenously). While still beating, these hearts were immersed in a modified Tyrode solution. The anterior papillary muscle with attached false tendon containing Purkinje fibers was removed from the right ventricle. A strip of tissue approximately 1 by 3 cm was removed from the right atrium. This strip was cut to include the superior portion of the right anterior crest. The endocardial surface of the atrial strip was used for study.

The muscles were pinned under slight tension to a paraffin block in a tissue bath of 15 ml volume. Tyrode solution equilibrated with 95% oxygen and 5% carbon dioxide flowed continuously through the bath. The composition of the solution in mmoles/liter was: NaCl 137, dextrose 5.5, KCl 2.7, CaCl₂ 2.7, MgCl₂ 0.5, NaH₂PO₄ 1.8, NaHCO₃ 12.0. Temperature of the bath was maintained at 35 to 37°C and remained constant during any experiment.

Microelectrodes were pulled from capillary tubing and filled with 3 M KCl. An indifferent electrode filled with 3 M KCl made contact with the fluid in the bath. Both electrodes were connected by chlorided silver spirals to the input of a Bioelectrics (type DS2C) cathode follower-amplifier which in turn was connected to the differential amplifier of a Tektronix type 502 oscilloscope. A 100 mv d-c calibrator was interposed between the indifferent electrode and the amplifier. Two identical assemblies made it possible to record simultaneously from two cells in the same preparation.

Rhythmic contractions at 95 per minute were maintained by applying suprathreshold square wave pulses of 5 msec duration. Single ventricular muscle cells, Purkinje cells or atrial muscle cells were impaled by advancing the microelectrode into the tissue until characteristic resting and action potentials were obtained. Only cells on the surface of the preparation were studied. The transmembrane resting and action potentials were monitored continuously on the type 502 Tektronix oscilloscope. Representative potentials were displayed on a Tektronix type 565 oscilloscope and photographed with a Grass kymograph camera.

Lee et al. found that an anesthetic mixture...
of 45% cyclopropane in oxygen caused spontaneous cardiac irregularities in dogs. At this concentration and at 38°C, the calculated tension of cyclopropane in plasma would be roughly 350 mm Hg. In the present experiments cardiac tissue was subjected to tensions of cyclopropane in this range. To obtain these values Tyrode solution was equilibrated at 38°C with known tensions of cyclopropane. The tension in the gas phase was calculated from a determination of the concentration of cyclopropane in this phase using a Burrell gas chromatograph. Since the gas and liquid phases were in equilibrium, this value was taken as the tension of cyclopropane in the liquid phase. The concentration in the liquid phase was determined by the method of Orcutt and Waters. From these data a graph of concentration vs. tension for cyclopropane in Tyrode solution was constructed.

In an open perfusion system, as employed here, gas is lost as the fluid circulates from the equilibration reservoir to the tissue bath. This loss is dependent in part on the perfusion rate. It was found that equilibrating reservoir Tyrode solution with a mixture containing 42 to 45% cyclopropane, 50 to 53% oxygen, and 5% carbon dioxide and perfusing the solution at a rate of 30 ml per minute in the tissue bath of 15 ml volume gave a concentration at the tissue of 6 to 8 vol% cyclopropane. From the concentration vs. tension plot previously determined concentrations of 6 to 8 vol% of cyclopropane were obtained with gas tensions of 300 to 400 mm Hg. Partial pressures of oxygen and carbon dioxide ranged from 275 to 410 and 38 to 46 mm Hg respectively. Oxygen and carbon dioxide tensions were measured by means of an Instrumentation Laboratories model 113 gas analyzer.

The effect of lowered oxygen tension was studied by replacing cyclopropane with nitrogen. This was done by equilibrating the reservoir Tyrode solution with a mixture of gas containing 35% oxygen, 60% nitrogen, and 5% carbon dioxide. Oxygen tensions in the bath were 5 to 75 mm Hg lower during this perfusion than during perfusion with the cyclopropane solution.

In the following discussion Tyrode solutions equilibrated with 95% oxygen and 5% carbon dioxide, the above cyclopropane gas mixture and the above nitrogen gas mixture will be referred to, respectively, as control-Tyrode, cyclopropane-Tyrode, and nitrogen-Tyrode solutions.

The general procedure of the experiment was as follows: Perfusion with control-Tyrode solution was maintained until penetration of a single Purkinje, ventricular or atrial cell was accomplished. Perfusion with cyclopropane-Tyrode solution was then started. Approximately two minutes were required for the solution to reach the tissue bath. Perfusion was maintained for 5 to 15 minutes and then changed back to either control-Tyrode solution or to nitrogen-Tyrode solution. Photographic records of action potentials were taken on clear base film before and at intervals during the test perfusions. These records were projected with a photographic enlarger onto a grid calibrated in millivolts and milliseconds. The method of analyzing action potentials was a modification of that used by Peterson and Fiegen for atrial cells. Figure 1 illustrates the methods used to obtain time and voltage measurements of membrane potentials of Purkinje fibers. Similar measurements were made on potentials from ventricular and atrial cells except that measurements of the slopes of phases 2 and 3 were not attempted. Only data from experiments in which the microelectrode remained in the same cell throughout the control and test series were used for statistical comparison. The statistical method used was Student's paired t-test.

**Results**

**Purkinje Fibers**

Twenty-three cells from 16 hearts were perfused with normal-Tyrode solution followed by cyclopropane-Tyrode solution. Data are summarized in table 1. Changes in contour of the action potential occurred consistently as shown in figure 2. The rate of repolarization during the plateau (phase 2) increased while
that during the period of rapid repolarization (phase 3) decreased. A quantitative measure of these changes was obtained by determining the slopes of lines tangent to these phases of repolarization. The slope of phase 2 increased significantly (mean increase 0.053 m/sec, \( P < 0.01 \)) while that of phase 3 decreased significantly (mean decrease 0.161 m/sec, \( P < 0.01 \)). Because of these changes the transition from phase 2 to phase 3 was not as abrupt as normal and the level of transmembrane potential during phase 2 and the initial part of phase 3 was at a higher (more negative) level during cyclopropane administration. Such changes resulted in a decrease in the time to repolarize to minus 60 millivolts. The total duration of the action potential lengthened largely due to the decrease in rate of repolarization during the terminal phase of repolarization. There were small but statistically significant decreases in resting potential, magnitude of the action potential and overshoot. Most of the cells studied showed some diastolic depolarization even though driven at a rate of 95/minute. An increase in diastolic depolarization occurred in 5 of 23 cells studied but the mean was not significant at the 99% level of confidence.

The possibility that these changes were caused by the relative hypoxia which accompanied cyclopropane administration was studied by perfusing the tissue with nitrogen-Tyrode solution in which oxygen tension was equal to or lower than that in cyclopropane-Tyrode solution. In 13 experiments cyclopropane perfusion preceded nitrogen and in 5 experiments the sequence was reversed. Results of a typical experiment are shown in figure 3. From this figure it is apparent that during perfusion with nitrogen-Tyrode the changes in contour of the action potential produced by cyclopropane returned to that present during normal-Tyrode perfusion. In experiments where nitrogen perfusion followed normal-Tyrode perfusion no readily apparent changes in contour of the action potential were noted and subsequent treatment with cyclopropane caused changes as described above. The data from all experiments in which the effects

<table>
<thead>
<tr>
<th>Resting potential, mV</th>
<th>Action potential, mV</th>
<th>Overshoot, mV</th>
<th>Time to reach -60 mV, msec</th>
<th>Duration of terminal phase, msec</th>
<th>Action potential slope phase 2, mV/sec</th>
<th>Slope phase 3, mV/sec</th>
<th>Diastolic depolarization, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control-Tyrode mean</td>
<td>95.1</td>
<td>232.7</td>
<td>133.5</td>
<td>234.4</td>
<td>0.195</td>
<td>0.811</td>
<td>0.822</td>
</tr>
<tr>
<td>Nitrogen-Tyrode mean</td>
<td>95.7</td>
<td>232.3</td>
<td>133.3</td>
<td>234.9</td>
<td>0.195</td>
<td>0.811</td>
<td>0.822</td>
</tr>
</tbody>
</table>

*Numbers in parentheses indicate the number of preparations studied. Confidence intervals were computed at the 99% level.*

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of nitrogen and cyclopropane were compared and are summarized in table 1. Statistically, highly significant differences were present in the slopes of phases 2 and 3, the time to repolarize to minus 60 millivolts, and in the duration of the terminal phase of repolarization. Resting potential, magnitude of the action potential, overshoot, total action potential duration and diastolic depolarization were not significantly different.

Further statistical analysis was utilized to determine whether hypoxia contributed significantly to the effects of cyclopropane on Purkinje fibers. The mean differences which were significant between the control to cyclopropane studies and the nitrogen to cyclopropane studies were compared using the unpaired t-test. Since the differences were not statistically highly significant it was concluded that the changes in membrane repolarization described above must be attributed to an action of cyclopropane on Purkinje fibers and not to coincident hypoxia.

VENTRICULAR MUSCLE FIBERS

Ventricular muscle fibers were perfused with control-Tyrode solution and transmembrane potentials were recorded. Perfusion with cyclopropane-Tyrode solution was then started and action potentials were recorded 3 to 10 minutes later. Data from 20 experiments in which the microelectrode remained in the same cell throughout both periods of perfusion are summarized in table 2. Although statistically highly significant decreases occurred in magnitude of the action potential, these diff-
FIGURE 3

Effects of cyclopropane-Tyrode and nitrogen-Tyrode solutions on the action potential of a ventricular cell (lower trace) and a Purkinje cell (upper trace). A: in control-Tyrode. B: after five minutes cyclopropane-Tyrode perfusion, oxygen tension 340 mm Hg, carbon dioxide tension 41 mm Hg, cyclopropane concentration 6.3 vol%. C: after six minutes nitrogen-Tyrode perfusion, oxygen tension 305 mm Hg, carbon dioxide tension 40 mm Hg. Time and voltage calibrations as indicated.

TABLE 2

Ventricular Fibers: Mean Values and Mean Differences Between the Action Potentials Obtained in Control-Tyrode and Cyclopropane-Tyrode Solutions and in Nitrogen-Tyrode and Cyclopropane-Tyrode Solutions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control-Tyrode mean</th>
<th>Cyclopropane-Tyrode mean</th>
<th>Mean difference and confidence interval (20)*</th>
<th>Nitrogen-Tyrode mean</th>
<th>Cyclopropane-Tyrode mean</th>
<th>Mean difference and confidence interval (18)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting potential, mv</td>
<td>93.0</td>
<td>90.7</td>
<td>$-3.3 \pm 1.9^\dagger$</td>
<td>88.1</td>
<td>88.7</td>
<td>$+0.6 \pm 1.2$</td>
</tr>
<tr>
<td>Action potential, mv</td>
<td>112.4</td>
<td>109.1</td>
<td>$-2.3 \pm 1.5^\dagger$</td>
<td>109.1</td>
<td>109.3</td>
<td>$+0.2 \pm 1.5$</td>
</tr>
<tr>
<td>Overshoot, mv</td>
<td>19.4</td>
<td>19.0</td>
<td>$-0.4 \pm 0.7$</td>
<td>21.0</td>
<td>20.6</td>
<td>$-0.4 \pm 0.9$</td>
</tr>
<tr>
<td>Time to reach —60 mv, msec</td>
<td>141.9</td>
<td>137.4</td>
<td>$-4.5 \pm 4.7$</td>
<td>137.3</td>
<td>141.6</td>
<td>$+4.3 \pm 5.1$</td>
</tr>
<tr>
<td>Duration of terminal phase of repolarization, msec</td>
<td>33.4</td>
<td>31.0</td>
<td>$-2.4 \pm 4.0$</td>
<td>26.6</td>
<td>23.0</td>
<td>$-3.6 \pm 2.9$</td>
</tr>
<tr>
<td>Action potential duration, msec</td>
<td>189.6</td>
<td>179.2</td>
<td>$-10.4 \pm 10.3^\dagger$</td>
<td>173.1</td>
<td>173.9</td>
<td>$+0.8 \pm 6.1$</td>
</tr>
</tbody>
</table>

*Numbers in parentheses indicate the number of preparations studied. Confidence intervals were computed at the 99% level.

$^\dagger$Indicates $P < 0.01$. 

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FERENCES were usually small and not readily apparent on the unmagnified record. Action potentials recorded during two such experiments are presented in figures 2 and 3. It can be noted that there is little change in contour of the ventricular action potential at a time when that of the Purkinje fiber shows a typical cyclopropane effect. The only readily detectable change is in the small, blunt termination of the upstroke of the action potential which decreased in size or disappeared during cyclopropane administration. In 7 of 18 experiments this was the only gross change noted in contour of the ventricular action potential.

The decreases in resting potential and in duration of the action potential occurred most frequently in experiments in which the tissue was subjected to two or more periods of cyclopropane treatment. These changes were very similar to those reported for hypoxia, and it seemed possible that they might be due either to hypoxia or to an accumulative effect of cyclopropane. These possibilities were studied by perfusing the tissue with nitrogen-Tyrode solution. In 13 experiments cyclopropane perfusion preceded nitrogen perfusion while in 5 experiments the order was reversed. Data from these experiments are summarized in table 2. When the differences in the action potentials recorded during each type of perfusion were compared, no statistically significant changes in contour of the action potential were found.
In 3 experiments lowered oxygen tension caused considerable depolarization and shortening of the duration of the action potential. Subsequent perfusion with cyclopropane-Tyrode solution containing a slightly higher oxygen tension partially reversed these effects (fig. 4). The results of these experiments indicate that hypoxia per se is probably responsible for any changes in contour of the ventricular action potential that might occur during cyclopropane administration.

**ATRIAL FIBERS**

Two different types of atrial cells could be identified from the contour of their action potentials. The action potential of one cell was similar to that of Purkinje fibers in that it possessed a large sharp spike and a long plateau (fig. 6A). Cells of this type were found on the endocardial surface in the superior portion of the right anterior crest in 9 of 11 hearts. Many impalements in this area revealed only this type of cell. When the electrode was moved several millimeters lateral to either side of the anterior crest, cells were encountered in which the action potential had the classical shape reported by others. It was possible to record from both types of cell in the same preparation (fig. 5). The effect of cyclopropane was studied on both. Each type of cell is discussed separately.

**PLATEAU CELLS**

The effects of cyclopropane on 10 cells of this type from 9 hearts were studied. In all experiments perfusion with normal-Tyrode solution was maintained until suitable action potentials were obtained. In 7 experiments cyclopropane-Tyrode perfusion then was started and continued for 5 to 15 minutes followed by perfusion with nitrogen-Tyrode for an additional 5 to 15 minutes. In 3 experiments nitrogen perfusion preceded cyclopropane perfusion. Records from one of the former experiments are in figure 6. During cyclopropane perfusion the rate of repolarization increased and the action potential shortened in duration. The plateau almost disappeared. Subsequent perfusion with nitrogen-Tyrode solution caused little change except for the reappearance of the small “hump” of depolarization immediately following the spike. When nitrogen-Tyrode solution followed normal-Tyrode solution the action potential shortened in duration similar to that shown in figure 6. Subsequent treatment with cyclopropane caused little further change in contour except that the hump on the plateau disappeared. Data from all experiments are summarized in table 3. While the contour of the action potential changed significantly between normal and cyclopropane perfusions, there were no statistically significant differences in the changes.
Effects of cyclopropane-Tyrode and nitrogen-Tyrode solutions on a plateau atrial cell. A: in control-Tyrode. B: after six minutes cyclopropane-Tyrode perfusion, oxygen tension 320 mm Hg, carbon dioxide tension 39 mm Hg, cyclopropane concentration 6.8 vol%. C: after eight minutes nitrogen-Tyrode perfusion, oxygen tension 295 mm Hg, carbon dioxide tension 41 mm Hg. D: after return to control-Tyrode for eight minutes. Time and voltage calibrations as indicated.

caused by cyclopropane as compared with those caused by nitrogen. Thus it is not possible to state whether cyclopropane exerts an effect on this type of atrial tissue.

NONPLATEAU ATRIAL CELLS

Seven cells from six hearts were studied. The procedure for studying these cells was similar to that outlined for plateau cells. Perfusion with cyclopropane-Tyrode solution caused an acceleration of repolarization and a shortening of the duration of the action potential (fig. 7). Subsequent perfusion with nitrogen-Tyrode solution did not alter these changes appreciably. When nitrogen perfusion followed normal-Tyrode solution the action potential shortened in duration and was not altered significantly by subsequent perfusion with cyclopropane-Tyrode solution. Data from these experiments are summarized in table 4. Again since no statistically significant differences are indicated between cyclopropane perfusion and nitrogen perfusion no statement can be made regarding the effects of cyclopropane on these atrial muscle fibers.

Discussion

Isolated tissues are ordinarily perfused with Tyrode solution equilibrated with 95% oxygen. At perfusion rates used in these experiments this gave an oxygen tension in the tissue bath of approximately 550 mm Hg. Although this is several times greater than that of arterial blood, a high tension is necessary to support ade-
When cyclopropane was added to the Tyrode solution, oxygen tension was decreased to approximately 300 mm Hg. It becomes necessary then to determine whether the effects noted were due to cyclopropane per se or were simply secondary to a relative hypoxia. In these experiments this was studied by testing the effects of perfusion with a Tyrode solution in which nitrogen replaced cyclopropane to the extent required to give equivalent oxygen tensions in the bath. Whether cyclopropane exerts an effect on ventricular fibers cannot be stated definitely.

The action of oxygen on tissue was studied adequately tissues deprived of a blood supply.

### TABLE 3

<table>
<thead>
<tr>
<th></th>
<th>Control-Tyrode mean</th>
<th>Cyclopropane-Tyrode mean</th>
<th>Mean difference and confidence interval (10)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting potential, mv</td>
<td>90.7</td>
<td>87.4</td>
<td>-3.3 ± 2.4</td>
</tr>
<tr>
<td>Action potential, mv</td>
<td>116.4</td>
<td>109.0</td>
<td>-7.4 ± 5.9†</td>
</tr>
<tr>
<td>Overshoot, mv</td>
<td>26.4</td>
<td>21.9</td>
<td>-4.5 ± 4.4†</td>
</tr>
<tr>
<td>Time to reach -60 mv, msec</td>
<td>145.4</td>
<td>126.1</td>
<td>-19.3 ± 12.4†</td>
</tr>
<tr>
<td>Duration of terminal phase of repolarization, msec</td>
<td>115.4</td>
<td>117.3</td>
<td>+1.9 ± 17.2†</td>
</tr>
<tr>
<td>Action potential duration, msec</td>
<td>286.5</td>
<td>268.6</td>
<td>-17.9 ± 16.7†</td>
</tr>
<tr>
<td>Slope phase 2, v/sec</td>
<td>0.238</td>
<td>0.711</td>
<td>-0.046 ± 0.092†</td>
</tr>
<tr>
<td>Slope phase 3, v/sec</td>
<td>0.757</td>
<td>0.631</td>
<td>-0.047 ± 0.185†</td>
</tr>
</tbody>
</table>

*Numbers in parentheses indicate the number of preparations studied. Confidence intervals were computed at the 99% level.† Indicates P < 0.01.

### Notations
- Control-Tyrode
- Cyclopropane-Tyrode
- Mean difference and confidence interval
- Nitrogen-Tyrode
- Cyclopropane-Tyrode
- Mean difference and confidence interval

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*Data from various sources.*
FIGURE 7

Effects of cyclopropane-Tyrode on atrial fibers. Upper trace is from a nonplateau fiber and lower trace is from a plateau fiber. A: in control-Tyrode. B: after four minutes cyclopropane-Tyrode perfusion, oxygen tension 312 mm Hg, carbon dioxide tension 42 mm Hg, cyclopropane concentration 6.1 vol%. D: after four minutes nitrogen-Tyrode perfusion, oxygen tension 304 mm Hg, carbon dioxide tension 39 mm Hg. C: after return to control-Tyrode perfusion for ten minutes. Line of zero potential difference for the lower cell is 25 mv below that of the upper cell. Time and voltage calibrations as indicated.

TABLE 4

Non-Plateau Type Atrial Fibers: Mean Values and Mean Differences Between the Action Potentials Obtained in Control-Tyrode and Cyclopropane-Tyrode Solutions and in Nitrogen-Tyrode and Cyclopropane-Tyrode Solutions

<table>
<thead>
<tr>
<th></th>
<th>Control-Tyrode mean</th>
<th>Cyclopropane-Tyrode mean</th>
<th>Mean confidence difference and interval (7)*</th>
<th>Nitrogen-Tyrode mean</th>
<th>Cyclopropane-Tyrode mean</th>
<th>Mean confidence difference and interval (7)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting potential, mv</td>
<td>92.7</td>
<td>89.9</td>
<td>-5.8 ± 3.9†</td>
<td>87.5</td>
<td>88.8</td>
<td>+1.3 ± 2.7</td>
</tr>
<tr>
<td>Action potential, mv</td>
<td>118.6</td>
<td>111.3</td>
<td>-7.3 ± 6.9†</td>
<td>112.2</td>
<td>113.7</td>
<td>+1.5 ± 4.2</td>
</tr>
<tr>
<td>Overshoot, mv</td>
<td>25.8</td>
<td>24.4</td>
<td>-1.4 ± 2.7†</td>
<td>24.6</td>
<td>24.8</td>
<td>+0.2 ± 1.8</td>
</tr>
<tr>
<td>Time to reach -60 mv, msec</td>
<td>113.7</td>
<td>74.9</td>
<td>-38.8 ± 21.4†</td>
<td>75.0</td>
<td>76.5</td>
<td>+1.5 ± 10.5</td>
</tr>
<tr>
<td>Duration of terminal phase of repolarization, msec</td>
<td>136.4</td>
<td>109.4</td>
<td>-27.0 ± 22.2†</td>
<td>136.3</td>
<td>120.2</td>
<td>-16.1 ± 15.8</td>
</tr>
<tr>
<td>Action potential duration, msec</td>
<td>290.0</td>
<td>214.6</td>
<td>-75.4 ± 59.5†</td>
<td>238.8</td>
<td>227.7</td>
<td>-11.1 ± 23.3</td>
</tr>
</tbody>
</table>

*Numbers in parentheses indicate the number of preparations studied. Confidence intervals were computed at the 99% level.
†Indicates P < 0.01.

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because repolarization was affected by reduction of the oxygen tension equivalent to that present during cyclopropane perfusion. A suggestion that cyclopropane has little effect on ventricular fibers is given by the experiments in which ventricular action potentials were unchanged at a time when simultaneously recorded Purkinje action potentials showed marked changes. Also in a recent study Levy et al.8 recorded ventricular action potentials in dogs anesthetized with cyclopropane. While interpretation of their records is difficult because of spontaneous changes in rate and rhythm, the contour of the ventricular action potential was similar to that reported by others in normal isolated preparations.10 The only effect of cyclopropane not attributable to hypoxia was loss of the small spiked termination of the upstroke of the action potential.

Results obtained in this study show that atrial tissue is sensitive to the level of oxygen tension present in our cyclopropane solution. Recently, Smith et al.7 reported an acceleration in rate of repolarization in nonplateau atrial fibers of the rabbit when subjected to cyclopropane. This effect resembles closely that obtained in the present study simply by lowering the oxygen tension to levels present in solutions which contain 6 to 7 vol% cyclopropane. Therefore it has not been demonstrated conclusively that cyclopropane accelerates repolarization of atrial muscle fibers. The only effect on atrial tissue caused by cyclopropane was loss of the small "hump" on phase 2 of the plateau type cell.

The changes in slopes of phase 2 and phase 3 of the action potential of Purkinje fibers show that cyclopropane exerts an effect on repolarization of this tissue. This finding may be important because of the relation between attainment of a certain degree of repolarization and the end of refractoriness. In this connection Weidmann has shown17 that cathodal stimuli can re-excite Purkinje tissue when the transmembrane potential repolarizes to between minus 58 and minus 62 millivolts. Also Hoffman and Kao18 have shown that action potentials propagated from the ventricle can elicit conducted responses in Purkinje fibers when the membrane potential repolarizes to between minus 55 and minus 65 millivolts. In the present experiments the time required to repolarize to minus 60 millivolts was shorter during cyclopropane administration. On the basis of the studies cited above, this finding may be interpreted to indicate a decrease in length of the functional refractory period in Purkinje fibers subjected to cyclopropane. The length of the refractory period is a determinant of cardiac irritability and shortening is cited as a contributing factor in the production and maintenance of re-entrant activity.19 Whether this effect contributes to the origin of ventricular arrhythmias sometimes associated with cyclopropane anesthesia is a matter of speculation.

The precise mechanisms which produce the changes in repolarization during cyclopropane administration are unknown. Even formation of a tentative hypothesis is difficult because of lack of information about the events which normally produce the plateau phase of the cardiac action potential. However, there is one condition which results in an effect like that of cyclopropane. An increase in extracellular calcium ion concentration from 2.7 to 10.8 mmoles/liter accelerates the rate of repolarization during phase 2 of the Purkinje fiber action potential while decreasing the rate of repolarization during phase 3.19 Whether there is a relation between the actions of calcium and cyclopropane has not been investigated in this study.

The plateau type atrial fibers described here (fig. 6A) are of interest because of the prominent plateau of the action potential. The action potential of certain fibers of the dog atrium20 and rabbit atrium21 show a plateau but it is not of the magnitude obtained in this study. It should be noted that the plateau is sensitive to a lowering of oxygen tension; a decrease from 500 to 300 mm Hg causing marked shortening or loss of the plateau. Whether these cells perform a function apart from contraction is unknown. Recently presented evidence suggests that spread of excitation through the atria of the dog occurs through specialized pathways.22, 28 The
action potential of these cells resembles in some ways that obtained in fibers of the ventricular conducting system. It is unknown whether these cells comprise a specialized conducting system in the atrium of the dog.

**Summary**

The effects of cyclopropane (6 to 8 vol% in Tyrode solution) and of hypoxia, on the transmembrane potentials of atrial, ventricular and Purkinje fibers of the dog heart, were studied. Action potentials were recorded during perfusion with solution equilibrated with 95% O₂ and 5% CO₂ (control-Tyrode), during cyclopropane-Tyrode perfusion and during perfusion with solution in which nitrogen replaced cyclopropane (nitrogen-Tyrode). Changes in transmembrane potential observed during cyclopropane-Tyrode treatment were considered to be due to an action of cyclopropane only if they recovered or failed to appear during nitrogen-Tyrode perfusion. By interpretation on this basis it could not be determined that cyclopropane exerted an effect on atrial or ventricular fibers. In Purkinje fibers cyclopropane caused a significant increase in rate of repolarization during the plateau (phase 2) while the rate during the period of rapid repolarization (phase 3) decreased. The time required to repolarize to minus 60 millivolts was shortened significantly while the durations of the terminal phase of repolarization and of the total action potential were lengthened. A relative hypoxia averaging 300 mm Hg accelerated repolarization and reduced resting potential of atrial cells. Ventricular cells were affected similarly but only after two or more periods of hypoxia. Purkinje fibers were shown to be most resistant to this level of hypoxia.

**Acknowledgment**

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Effects of Cyclopropane and of Hypoxia on Transmembrane Potentials of Atrial, Ventricular and Purkinje Fibers
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