Prostigmine Inhibition of Ventricular Fibrillation in the Hypothermic Dog

By A. V. Montgomery, Ph.D., Arthur E. Prevedel, M.D., and Henry Swan, M.D.

This study shows that when prostigmine is given via coronary perfusion in the hypothermic dog, cardiac surgery can be performed without ventricular fibrillation resulting. This action of prostigmine seems to be due to an accumulation of acetylcholine, since prostigmine's antifibrillatory action can be reproduced by a continuous coronary perfusion of acetylcholine or by stimulation of the vagus nerve. A possible relationship between potassium and ventricular fibrillation is discussed.

Bigelow and co-workers have shown that the metabolic rate of dogs whose body temperature has been cooled to 20 C. rectal temperature is only about 15 per cent of control. This circumstance moved Bigelow and other coworkers to deliberately occlude circulation for periods of 15 minutes without observable damage to the heart or central nervous system in the surviving animals. However, about half of his animals died of ventricular fibrillation during the experiment.

Several workers have attested to the increased irritability of the ventricles in the hypothermic dog, but to date the explanation for this observation has been elusive. It appears clear that cardiac hypoxia, when the circulation is intact in the dog at 20 C. or above, does not seem to be a major factor. Penrod has shown that the arteriovenous oxygen difference across the coronary circulation remains normal until the rectal temperature falls below 20 C. Lower temperatures resulted in a rapid decrease in the A-V oxygen difference. This observation has been confirmed and extended recently by Edwards and associates.

In a search for other factors which might contribute to the increased irritability of hypothermia, Swan and some of his colleagues studied the plasma and thiocyanate volumes, blood pH, and sodium, potassium and chloride of the plasma in the normothermic hyperventilated animal and in the hypothermic animal under the influence of both hyperventilation and hypoventilation. They also reported that when animals were cooled to a rectal temperature of 25 C. and inflow occlusion to the heart was instituted, many animals incurred ventricular fibrillation, especially upon the release of the occlusion. However, the incidence of fibrillation was a function of ventilatory rate: in the hypoventilated group it was 50 per cent; in the hyperventilated group, 8 per cent.

The only recognized difference in the composition of the blood between these two groups was the pH. In both groups the plasma potassium was consistently low; it was felt likely, however, that intracellular differences might exist, and that an inverse relation between hydrogen ion concentration and intracellular potassium in the hypothermic dog might be present. Although it was found that in the hyperventilated hypothermic animal the loss of extracellular potassium was not accounted for by renal excretion of the ion, the difference was quantitatively small. This was interpreted to mean that either intracellular potassium remained the same or was increased slightly. Subsequently, somewhat more convincing evidence was obtained in the opposite direction in hypoventilated animals. Unpublished observations from this laboratory show that in the hypoventilated hypothermic dog

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the renal excretion of potassium is several times greater than would be required to account for the loss of extracellular potassium. It is felt that this observation indicates a loss of intracellular potassium in the hypothermic animal with a low blood pH.

There is another apparent relationship between fibrillation and potassium. Swan and associates found that the fibrillating hypothermic heart could rarely be converted to a normal rhythm by massage and electric shock. However, when the coronary arteries of the fibrillating heart were perfused with a potassium chloride solution (1 mEq. per milliliter) the fibrillation could usually be converted directly or by use of electric shock.

The above observations seemed to fit into a general theory previously advanced by Hooker. He had found that ventricular fibrillation in the isolated perfused heart could be converted by the addition of potassium to the perfusion medium. He also found that during ventricular fibrillation the heart lost potassium to the perfusion medium. From these observations he suggested that the potassium added to the perfusion fluid caused, by chemical mass action, the fibrillating myocardiun to make up its potassium deficiency and by so doing restored normal ventricular rhythm.

The above observations led us to explore the effect of certain agents known to have an effect on potassium distribution. Grieg and coworkers have described such an effect of acetylcholine on the isolated guinea pig heart. Unpublished observations by Holmes and Montgomery have shown that potassium movement between the intracellular and extracellular spaces can be affected by acetylcholine and cholinesterase inhibitors. The current report describes certain observations on the effect of prostigmine, acetylcholine, and stimulation of the vagus nerve on ventricular fibrillation in the hypothermic dog.

**Methods**

Two groups of experiments are presented: those concerned with the effect of certain drugs or procedures on ventricular fibrillation in hypothermia, and those concerned with the arteriovenous potassium difference across the coronary circulation during ventricular fibrillation or various degrees of ventilation during hypothermia. In the first group, two types of stimuli were used for the induction of ventricular fibrillation: ventricular incision and catheterization of the coronary sinus during inflow and outflow occlusion of the heart in the dog at 25 C. rectal temperature. The influence of prostigmine, acetylcholine, and vagal stimulation on the effect of these stimuli was studied.

In the initial experiments prostigmine methylsulfate (0.05 ml. per kilogram of a 1:4000 solution) was given intravenously five minutes before circulation was occluded and ventriculotomy performed. Later prostigmine was administered by a technic which we have termed coronary perfusion. This mode of administration was as follows. The azygos vein was ligated and the superior and inferior caval venae occluded. The ascending aorta was occluded by a Potts clamp. The outflow occlusion was applied about 15 seconds after the inflow to prevent acute dilatation of the heart. Then prostigmine (1:4000) was injected into the aorta proximally to the clamp so that as the heart contracted the drug was perfused through the coronary circulation. If the drug was used only after fibrillation had ensued, cardiac massage was instituted to force the drug through the coronaries. When acetylcholine was used, its short action required continuous administration throughout the period of cardiac manipulation and release of occlusion. Both drugs were administered slowly until the heart rate was reduced to between 10 to 25 beats per minute. An initial 1 ml. injection of prostigmine was given and the heart rate was determined. Prostigmine was then given, 0.5 ml. at a time, until the desired rate was achieved. Acetylcholine was administered at a rate of 0.5 mg. per minute until the desired rate was achieved, then it was given more slowly throughout the procedure in quantities just sufficient to maintain the heart rate between 10 to 25 beats per minute. When vagal stimulation was being used to increase the threshold to ventricular fibrillation, the right vagus nerve was stimulated with a square wave of voltage varying from 25 to 40 volts and at a frequency of from 8 to 25 stimuli per second. These variables were adjusted to give the same heart rate that was obtained in the prostigmine and acetylcholine experiments. Usually the frequency of stimulation had to be gradually increased during the procedure in order to maintain this slow rate.

All animals were anesthetized with 35 mg. per kilogram of sodium pentobarbital given intravenously before immersion in an ice bath. More pentobarbital was administered if any observable shivering occurred. When the animal was placed in the ice, it was connected to a respirator supplied with pure oxygen.

Cannulation of the coronary sinus was accomplished in the following manner. A small transverse skin incision was made to expose the external jugular
vein. A no. 18 polyethylene catheter with a small curved glass tip was placed into the vein and pushed into the superior vena cava. When vagal stimulation was to be performed, the right or both vagus nerves were isolated. A right thoracotomy was made through the fifth intercostal space and upon opening the chest the azygos vein was ligated. Both cavae were isolated and the pericardial sac opened. Both cavae were then occluded with umbilical tape and 15 seconds later the ascending aorta was occluded just distal to the ostia of the coronary arteries. The right atrium was then opened widely and the catheter tip was inserted into the coronary sinus. A suture into the posterior wall of the atrium held the catheter tip in place. The atrium was filled with saline and the opening and the incision closed with a curved Potts clamp. The aortic occlusion was released first, followed by simultaneous release of both cavae. The total occlusion time was from three to four minutes. If vagal stimulation was being used to prevent fibrillation during the procedure, the nerve was stimulated until after the atrium had been closed with a continuous mattress stitch.

Blood samples were drawn periodically from the coronary sinus and from the femoral artery. These samples were analyzed for potassium by a Janke flame photometer.

Results

Table 1 reveals data obtained from experiments on 65 dogs. All of these animals were hyperventilated with oxygen and were cooled to 25 C. The stimulus for ventricular fibrillation was a 3 to 4 cm. incision into the right ventricle. It is seen that in the control series of animals without pretreatment 23 out of 23 animals incurred ventricular fibrillation following ventriculotomy.

Prostigmine 1:4000 (0.05 cc. per kilogram) was administered intravenously five minutes before circulatory arrest to 15 animals. Only seven, or about 50 per cent, of these animals developed ventricular fibrillation following ventriculotomy. The remaining eight animals are still living. Five of the animals that fibrillated were converted to a normal sinus rhythm by cardiac massage and electric shock. All of the converted animals also survived the entire procedure.

The next experiments concerning the effect of prostigmine had a dual purpose. The first was of a practical nature. We wanted to see if a higher concentration of prostigmine locally to the heart might decrease the incidence of ventricular fibrillation without the total dose administered being in the lethal range of prostigmine. The second purpose was to determine whether the antifibrillatory effect was a local one rather than a general systemic effect.

With these ideas in mind, 1 to 3 ml. of 1:4000 solution of prostigmine was administered by coronary perfusion as described above. The clearly visible effects of prostigmine administered in this manner are quite dramatic. Even though inflow to the heart is occluded, the myocardium becomes pink, the diastolic volume is reduced, and the contractions become more forceful. Table 1 shows that of the 16 animals treated in this manner ventriculotomy failed to induce ventricular fibrillation in a single instance. In addition to the routine right ventriculotomy, eight of these animals survived a left ventriculotomy and the creation of an interventricular septal defect all in the same operative procedure. All of these animals warmed at the normal rate and lived until sacrificed weeks later.

Since the cholinesterase enzyme normally hydrolyzes acetylcholine, the effects of cholinesterase inhibitors can often be explained on the basis of an accumulation of acetylcholine. If such is the mode of action of the antifibrillatory effect of prostigmine, one would expect a continuous perfusion of acetylcholine through the coronaries to duplicate the effect. Table 1 shows that in five animals given such perfusion, acetylcholine did prevent ventricular fibrillation following ventriculotomy.

Since the vagus nerve is thought to influence

<table>
<thead>
<tr>
<th>Method</th>
<th>No. of Dogs</th>
<th>Ventric. Fibrill.</th>
<th>Resuscitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23</td>
<td>23</td>
<td>0 of 23</td>
</tr>
<tr>
<td>Prostigmine in open circulation</td>
<td>15</td>
<td>7</td>
<td>5 of 5 attempts</td>
</tr>
<tr>
<td>Prostigmine by coronary perfusion</td>
<td>16</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>Acetylcholine by coronary perfusion</td>
<td>5</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>Continuous stimulation of right vagus</td>
<td>6</td>
<td>2</td>
<td>2 of 2 attempts</td>
</tr>
</tbody>
</table>
the heart by the release of acetylcholine, one would expect that stimulation of the peripheral stump of the vagus would mimic the antifibrillatory effect of prostigmine and acetylcholine. In six experiments, such vagal stimulation protected four animals from fibrillation, while the remaining two were easily converted to normal rhythm by massage and electric shock.

It was desired to catheterize the coronary sinus in order to determine coronary sinus potassium concentration. In the cold dog this was found to be a potent stimulus to fibrillation. Table 2 shows that 11 out of 12 dogs developed fibrillation with the catheterization procedure. None of these animals could be converted to a normal rhythm by cardiac massage and electric shock. However, after coronary perfusion of prostigmine all 11 animals were readily converted by electric shock. This table also shows that coronary perfusion of prostigmine prior to catheterization protected another 21 dogs against ventricular fibrillation. Table 2 also reveals that stimulation of the vagus nerve during the catheterization protected all 10 against fibrillation.

On the basis of observations previously reported,7 it was suspected that blood pH influenced the concentration of potassium in the myocardium. Since it was shown7 that hyperventilation reduces the incidence of ventricular fibrillation in the hypothermic dog as compared with the hypoventilated animal, the effect of pH on potassium metabolism of the heart receives greater importance. In order to shed light on this point we examined the arteriovenous difference in plasma potassium concentration across the coronary circu-

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TABLE 2.—Ventricular Fibrillation in Hypothermic Dogs Using Coronary Sinus Catheterization as Stimulus

<table>
<thead>
<tr>
<th>No. of Dogs</th>
<th>Ventric. Fibrill.</th>
<th>Resuscitation (with Prostigmine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>Prostigmine by coronary perfusion</td>
<td>21</td>
<td>0 —</td>
</tr>
<tr>
<td>Continuous stimulation of right vagus</td>
<td>10</td>
<td>0 —</td>
</tr>
</tbody>
</table>

[FIG. 1. Arteriovenous difference in plasma potassium concentration across the hypothermic myocardium, 25 C. during variations in blood pH controlled by respiration. (Simultaneous femoral artery and coronary sinus blood samples.)]
TABLE 3.—Serum Potassium Level of Coronary Sinus Blood before and during Ventricular Fibrillation

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Before Fibrillation (mEq./L.)</th>
<th>During Fibrillation (mEq./L.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-1</td>
<td>2.56</td>
<td>7.42</td>
</tr>
<tr>
<td>F-2</td>
<td>3.02</td>
<td>8.50</td>
</tr>
<tr>
<td>F-3</td>
<td>2.67</td>
<td>5.64</td>
</tr>
<tr>
<td>F-4</td>
<td>3.13</td>
<td>9.69</td>
</tr>
<tr>
<td>F-5</td>
<td>2.01</td>
<td>6.15</td>
</tr>
<tr>
<td>F-6</td>
<td>2.15</td>
<td>8.30</td>
</tr>
<tr>
<td>F-7</td>
<td>2.68</td>
<td>7.53</td>
</tr>
<tr>
<td>Mean</td>
<td>2.17</td>
<td>7.61</td>
</tr>
</tbody>
</table>

in table 3. The mean coronary sinus potassium before fibrillation was 2.6 mEq. per liter and 7.5 mEq. per liter during fibrillation. In every animal more potassium was leaving the heart during fibrillation than before.

**Discussion**

The results reported here show that the administration of prostigmine to the hypothermic dog has a pronounced antifibrillatory effect. When this drug is given by coronary perfusion, it is more effective. Since prostigmine is a cholinesterase inhibitor, one might expect that the antifibrillatory effect of prostigmine is secondary to an accumulation of acetylcholine. Evidence for this idea receives support from two other observations in the present report. Infusion of acetylcholine through the coronaries inhibits ventricular fibrillation, as does also an increase in endogenous acetylcholine by vagal stimulation. Prostigmine appears to be the drug of choice for practical application. The use of this agent gives promise of being a potent weapon for the prevention and management of the ventricular fibrillation associated with hypothermia. In our hospital we are now giving this drug clinical trial.

In the present state of knowledge, it is impossible to describe the underlying phenomena at the cellular level which influence ventricular fibrillation or to portray the mechanism by which acetylcholine prevents fibrillation in the hypothermic dog. However, there is a growing body of evidence which strongly implicates potassium distribution as being related to this phenomenon. Howell showed long ago that stimulation of the vagus nerve caused a loss of potassium from the heart. Hooker has shown that during ventricular fibrillation the isolated perfused heart is losing potassium. The results presented here show that the same can be said of ventricular fibrillation in the hypothermic dog. Bigelow reported a 50 per cent incidence of ventricular fibrillation following release of a 15 minute inflow occlusion in the hypothermic dog. The pH of his animals was low because they were breathing a gas containing a high concentration of carbon dioxide. Swan and associates reported the same incidence of ventricular fibrillation when the pH was depressed by hyperventilation of the animal. However, the incidence of fibrillation was reduced to 8 per cent when the animals were hyperventilated.

The results reported here suggest that the high incidence of fibrillation associated with hyperventilation in hypothermia occurs under the conditions of a myocardium which has gained potassium. In short it appears that an acidotic, hypothermic myocardium gains potassium and is subject to fibrillation; but whenever the myocardium, warm or cold, acidotic or alkalotic, enters fibrillation, potassium is released from the heart.

Another suggestive line of investigation relating potassium to fibrillation was begun by Brown and Miller who found a high incidence of fibrillation in dogs suddenly changed from a breathing mixture containing a high concentration of carbon dioxide to room air. Young, Sealy, and Harris have recently focused attention on the changes occurring during the first few minutes of normal respiration in similar experiments. They found that the serum potassium rose as the pH fell when the animals were being ventilated with 20 per cent carbon dioxide. After the animals had been breathing this gas mixture for two to four hours, they were suddenly allowed to breathe air. He found that this maneuver resulted in a rapid rise in pH, but, more importantly, the serum potassium at first rose abruptly to even higher levels during the first few minutes before beginning a fall toward normal. His animals incurred ventricular fibrillation during the time of the abrupt rise
in serum potassium. Intravenous injection of 3 per cent sodium chloride solution reduced the potassium rise and prevented ventricular fibrillation. The cause of the temporary but severe dissociation of potassium and pH during these first few minutes is unknown.

Grieg and associates have made observations on the rate of movement of potassium across the myocardial membrane of the guinea pig. They have reported that acetylcholine increases the rate of movement of potassium across cell membranes, the direction of movement depending on the direction of deviation from the normal potassium gradient across the membrane. On the basis of these observations, one would predict that if serum potassium were suddenly lowered, thus increasing the gradient, potassium would tend to move out of the cell. Acetylcholine would accelerate the shift. This relationship has not been demonstrated in the cold animal.

The varied data presented above certainly do not allow a definitive description of the relation between potassium distribution and ventricular fibrillation. However, taken as a whole they strongly suggest that such a relationship exists. Much further work needs to be done in this area of investigation.

At the integrative level, however (that is, in the area of mechanisms impinging on and regulating cell activity), at least one working hypothesis can be presented. It is our intention to submit this hypothesis to experimental evaluation in the immediate future. Adrenaline and probably sympathetic impulses to the heart may induce ventricular fibrillation or potentiate other factors which induce fibrillation. There is evidence in the literature which indicates that under hypothermia the heart is receiving predominantly sympathetic impulses. Bigelow has estimated the peripheral resistance in hypothermic animals by the simultaneous determination of arterial pressure and cardiac output. This observation suggests a strong sympathetic activity. In the experiments reported here concerning vagal stimulation, it was observed that section of the vagus nerves does not lead to a cardiac acceleration. This finding confirms a similar observation by Cookson and DiPalma.

This could mean either that in hypothermia there is an absence of vagal cardiac impulses or that the heart is no longer sensitive to acetylcholine released at the vagal endings. Two observations militate against the latter view: electrical stimulation of the peripheral vagus and acetylcholine perfusion cause cardiac slowing in the cold heart.

The above considerations lead us to suggest the following possible mechanism. The low systemic arterial pressure in hypothermia activates the carotid sinus reflex, resulting in a lack of vagal impulses to the heart, an increase in sympathetic impulses to the heart, and an intense vasoconstriction mediated by sympathetic fibers. From this point of view the antifibrillatory effects of proctigmine, acetylcholine and vagal stimulation become more meaningful. Each procedure increases a depressed parasympathetic influence on the cold heart, thus restoring a more nearly normal balance of sympathetic and parasympathetic effects.

Summary
1. Prostigmine has a marked antifibrillatory effect upon the ventricular myocardium of the hypothermic dog.
2. Acetylcholine or vagus nerve stimulation also has this effect.
3. The hypothermic myocardium during respiratory acidosis gains potassium.
4. The hypothermic myocardium during respiratory alkalosis maintains potassium balance.
5. The hypothermic myocardium in ventricular fibrillation loses potassium.

Sumario Español
1. La prostigmina tiene un efecto antifibrilatorio marcado en el miocardio ventricular del perro hipotérmico.
2. La acetilcolina o la estimulación del vago también tienen este efecto.
3. El miocardio hipotérmico gana potasio durante la acidosis respiratoria.
4. El miocardio hipotérmico mantiene el balance de potasio durante la alcalosis respiratoria.
5. El miocardio hipotérmico pierde potasio durante la fibrilación ventricular.

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Prostigmine Inhibition of Ventricular Fibrillation in the Hypothermic Dog
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