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Effect of ischemia of lower esophagus and esophagogastric junction on canine esophageal motor function

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The etiology of esophageal achalasia is unknown. However, the common finding of a decreased number or an absence of ganglion cells in Auerbach’s plexus has long been considered as etiologically significant in this disease. It is still not known whether this is a primary change or whether it is secondary to disease of the extrinsic esophageal nerve supply. The purpose of this study was to develop a technique that would damage or destroy the ganglion cells in Auerbach’s plexus of the lower part of the esophagus and gastroesophageal junction and to determine the effects on esophageal function. Ischemia was selected as the means of damaging the ganglion cells because neural tissue is very sensitive to anoxia.

Methods

Fifteen adult mongrel dogs, weighing 12 to 16 kilograms, were used. The animals were anesthetized with pentobarbital sodium, 25 mg. per kilogram of body weight, and were ventilated artificially with air through a cuffed endotracheal tube. Normal body temperature was maintained throughout the procedure by placing the animals on a heating pad.

With sterile techniques, the lower end of the esophagus and the upper portion of the stomach were mobilized through a left thoracoabdominal incision after division of the phrenoesophageal ligament. After the left gastric artery and vein had been dissected and separated from the remainder of the vascular pedicle, the lower 10 cm. of the esophagus and the upper portion of the stomach were isolated by placing clamps across the esophagus below the inferior pulmonary vein and across the stomach 5 cm. below the gastroesophageal junction; another small clamp was placed on the remnant of the vascular pedicle (Fig. 1). Tyrode’s solution at body temperature was perfused through the left gastric artery at a pressure of 130 cm. of water until the isolated area became white (Fig. 2), after which it was stopped and the left gastric vein was temporarily occluded. Four hours later the clamps were removed and the left gastric artery ligated. Silver clips were applied to

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the upper margins of the esophageal hiatus and to the limits of the perfused segment of bowel for later radiographic identification. Penicillin, 500,000 units, was given daily by intramuscular injection for 5 days. After the operation the animals were fed milk until they could eat a normal kennel diet.

At monthly intervals after the procedure, cineradiography and esophageal motility studies were performed with the animals unanesthetized. The technique of esophageal manometry as performed at this institution has been described previously. At the time of death, the perfused portion of esophagus and stomach was removed and examined. Histologic sections were made and stained by various techniques. Longitudinal sections at the gastroesophageal junction were stained by hematoxylin and eosin, cresyl violet, Bielschowsky's, and van Gieson's methods. These sections were used to assess whether ganglion cells and other tissues had been damaged, and were valuable in showing detailed cytologic changes in individual ganglion cells. To assess the actual numbers of ganglion cells more accurately, a specimen of muscle, consisting of the upper portion of the inferior esophageal sphincter and its junction with the lower end of the striated muscle of esophagus, was cut parallel to the

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Fig. 1. Ischemic portion extends from below inferior pulmonary vein (IPV) to upper portion of stomach. Blood was removed from this portion by perfusion with Tyrode's solution through left gastric artery (LGA). AA = aortic arch.

Fig. 2. Operative procedure. Isolated upper portion of stomach and lower esophagus are seen through a left thoracoabdominal incision. Cannula was inserted into left gastric artery (LGA); left gastric vein (LGV) became translucent, and a bulldog clamp occluded remainder of vascular pedicle. Double clamps on left were placed on upper portion of stomach.
mucosa and in the same plane as Auerbach's plexus with a thickness of 175 microns. These sections were treated by an indoxyl esterase technique which preferentially stained the mitochondria of the ganglion cells blue. The total number of ganglion cells in a block of tissue could be assessed easily and reliably under low magnification.

Results

Three of the 15 dogs died of pulmonary infection within a week of operation and were not available for physiologic investi-

<table>
<thead>
<tr>
<th>Dog</th>
<th>Weeks from operation to death</th>
<th>Weeks of esophageal dilatation</th>
<th>Ganglion cell changes</th>
<th>Reduction in number of cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1/2</td>
<td>—</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>1/4</td>
<td>—</td>
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</tr>
<tr>
<td>3</td>
<td>1</td>
<td>—</td>
<td>Mild</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>2</td>
<td>Marked</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>4</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>6</td>
<td>15</td>
<td>2</td>
<td>Mild</td>
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</tr>
<tr>
<td>7</td>
<td>16</td>
<td>4</td>
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<tr>
<td>8</td>
<td>18</td>
<td>10</td>
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<td>Moderate</td>
</tr>
<tr>
<td>9</td>
<td>18</td>
<td>3</td>
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</tr>
<tr>
<td>10</td>
<td>19</td>
<td>16</td>
<td>Mild</td>
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</tr>
<tr>
<td>11</td>
<td>27</td>
<td>2</td>
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</tr>
<tr>
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<td>32</td>
<td>32</td>
<td>Marked</td>
<td>Marked</td>
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<tr>
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<td>38</td>
<td>20</td>
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</tr>
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<td>48</td>
<td>32</td>
<td>Mild</td>
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</tr>
<tr>
<td>15</td>
<td>48</td>
<td>43</td>
<td>Moderate</td>
<td>Mild</td>
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</tbody>
</table>

Fig. 3. Esophageal roentgenograms made 1 month (A) and 3 months (B) after operation. Dilatation of esophagus affected both perfused and intact portions. Lower four clips were placed on esophageal hiatus, and top two mark upper end of perfused portion.
Fig. 4. Two postoperative patterns of pressure and mucosal potential difference (PD) at gastroesophageal junction. After ischemia, upper recording shows low sphincteric pressure and lower shows more nearly normal pressure.

Fig. 5. Responses of canine esophagus and gastroesophageal sphincter to swallowing before and after ischemia. In intact animal, relaxation and contraction of sphincter were seen. One month after operation there was no relaxation of sphincter and there were weak simultaneous contractions in body of esophagus. Ten months after operation, weak peristalsis returned.
gations. The remaining 12 survived and were usually able to eat a normal kennel diet within 2 to 3 weeks.

**Cinefluoroscopy.** Cinefluoroscopy revealed that the esophagus was dilated for a varying period in all the dogs studied (Table I and Fig. 3) but returned to normal except in 1 which was killed at 32 weeks. The greater the initial difficulty in swallowing, the longer was the period of dilatation of the esophagus. When gross dilatation was present, the esophagus would contain up to 150 ml. of a barium sulfate solution, whereas, in the normal dog, 5 to 10 ml. in the body of the esophagus is sufficient to induce a peristaltic contraction that empties the lumen. It was possible to assess the degree of dilatation by measuring the residue contained in the esophagus, and it was noted that the amount in the body necessary to produce relaxation of the sphincter gradually reduced over the months but never returned to normal values. Stricture formation, either at the gastroesophageal junction or where the clamps had been applied, was excluded by following the progress of radiopaque rubber balloons with a circumference of 6.3 cm. down the esophagus into the stomach.

Fluoroscopic examination revealed gross disturbances of esophageal motor function in the early postoperative period, which returned toward normal later. The peristaltic wave was normal in the upper portion of the dilated esophagus but did not pass through the region which had been rendered temporarily ischemic, and the sphincter rarely relaxed in response to a swallow. Later in the postoperative period, peristalsis returned in the perfused portion so that the majority of the peristaltic sequences were followed by relaxation of the sphincter, but the esophagus would not be completely cleared of its contents. Finally, the peristaltic wave became more powerful and was effective in completely clearing the esophagus of all the ingested barium.

**Esophageal manometry.** Studies of the esophagus at rest usually revealed a slight decrease in maximal pressure at the gastroesophageal junction and an increase of pressure in the body of the esophagus (Fig. 4). Occasionally the sphincteric pressures remained low throughout the period of observation, but they more often returned to normal. In the early postoperative period, the response to swallowing consisted of weak simultaneous contractions in the perfused part and absence of sphincteric relaxation (Fig. 5). Later in the postoperative period the contractions in the lower esophagus became peristaltic and more vigorous. However, in this same period, even when peristalsis in the body of the esophagus had returned to normal, the sphincter often failed to relax and occasionally responded with a premature contraction. The majority of animals eventually had normal esophageal motility, but in a few there was a residual reduction in the amplitude of the peristaltic wave in the portion which had been rendered temporarily ischemic. The timing of these changes in esophageal caliber and

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**Fig. 6.** Gastroesophageal junction after ischemia showed no stricture and a normal gastroesophageal junction.
Fig. 7. Longitudinal sections (10μ thick) through canine esophageal wall. (Cresyl violet technique; ×225.) A, Intact animal. Normal ganglion cells. B, One week after ischemia. Ganglion cell changes consist of peripheral accumulation of Nissl's granules and disruption of some cells. C, Eleven months after ischemia. Only one abnormal ganglion cell can be seen (arrow).
function was variable and no precise pattern could be identified.

Anatomic studies. The gross appearance of the gastroesophageal junction was normal, and none of the dogs had a stricture at the lower end of the esophagus or at the upper end of the perfused portion where a clamp had been applied (Fig. 6).

The histologic changes in the individual ganglion cells were shown best by the cresyl violet technique (Fig. 7). In the early postoperative period the shape of the cell was distorted, and the Nissl granules in the cytoplasm accumulated at the periphery, leaving a relatively clear area surrounding the nucleus. The nucleus lost its normal oval shape and often became crenated. Occasional double nucleoli also were seen. In the later postoperative period, clumping of Nissl granules in the cytoplasm, disruption of the cell margin, or contraction of the cell with loss of the nucleus occurred. In those animals with a decrease in the number of the ganglion cells, the cells remaining were usually abnormal.

The number of ganglion cells was assessed after treating the sections, 175 microns thick, with indoxyl esterase (Fig. 8). In 5 of the 14 dogs, the number was less than normal. In 2 dogs that had a dilated esophagus for 32 weeks, the decrease was marked. Cells were stained even when the nucleus had disappeared and the cytoplasm had disrupted, but disappearance of the cell as demonstrated by this technique usually took 4 months.

Discussion

Various techniques have been used to destroy the ganglion cells in Auerbach's plexus. Carbon dioxide snow, irradiation, and phenolic derivatives destroy ganglion cells but also damage smooth muscle. Although motor function has been disturbed by pharmacologic agents, such as nicotine, pilocarpine, and di-isopropyl fluorophosphate, permanent damage to the neural tissue does not usually occur.

Use of ischemia has proved a good technique for destroying ganglion cells selectively. This was first described by Cannon and Burket in 1913. Their technique was based on the concept that when the intestinal wall, which consists of epithelium, connective tissue, muscle, and nerve, was damaged by anoxia, the neural tissue was affected the most and was incapable of regenerating. After a period of ischemia, the other tissues recovered but the neural tissue disappeared. They compressed the small intestine of the dog between glass plates for 3½ hours and observed motility distur-
bances and histologic changes in the ganglion cells of Auerbach's plexus over the next 30 days. The critical period of 3/2 to 4 hours of ischemia was confirmed by Kessler and Linden on the small bowel of the dog. Ischemia of the lower end of the esophagus by means of compression between plates successfully destroyed ganglion cells in the dog and in the cat and was accompanied by radiographic evidence of abnormal esophageal motility.

A more physiologic technique of inducing ischemia was described by Hukuhara and associates, who perfused a segment of canine small intestine with Tyrode's solution through its blood supply. By this technique, Hukuhara was successful in destroying ganglion cells and this finding has been reproduced by others. One attempt to use this technique on the colon of the dog did not damage the ganglion cells. Okamoto and co-workers applied the technique of Hukuhara and associates to the lower end of the esophagus and upper portion of the stomach by perfusing the left gastric artery with Tyrode's solution. They shortened the critical period of anoxia from 4 hours to 1 hour by adding a weak solution of mercuric chloride. Ganglion cell changes and a permanently dilated esophagus were obtained in the majority of the dogs, but esophageal strictures may have been produced.

The present method is a modification of the technique of Okamoto and associates; ischemia of 4 hours' duration was obtained by perfusing with Tyrode's solution alone, because the effect of mercuric chloride is not fully known. Also, by using a thoraco-abdominal approach, a 10 cm. segment of esophagus rather than a 5 cm. segment was made ischemic. There were no gross alterations in the perfused segment, but definite, although temporary, alterations in esophageal motor function were obtained. The esophagus became dilated in both the perfused and the intact portions. Dilatation occurred because the lower end of the esophagus contracted weakly and without peristalsis, and the sphincter failed to relax and contract in a correct time sequence. After the early postoperative period, which usually lasted at least a few weeks, the dilatation decreased. This was accompanied by return of muscular power and peristalsis in the lower esophagus and by resumption of the normal time sequence of relaxation and contraction of the sphincter. In the late postoperative period the motor function became normal in all dogs except one.

Accompanying these motor disturbances were definite cytologic changes in the ganglion cells of Auerbach's plexus in 11 dogs. No actual cell counts were made, but a comparative estimate of cell numbers between normal and the experimental dogs was made. In 5 of the 12 survivors the numbers were definitely reduced. This was always true of animals that lived more than 4 months with a dilated esophagus, suggesting a correlation between the severity of the histologic damage and esophageal motor disturbance.

However, motor function was most severely deranged in the early postoperative period, whereas the loss of ganglion cells was most severe in the later period. One can only postulate reasons for this. Light microscopy revealed no evidence of esophageal muscle abnormality, except edema and red cell infiltration during the first few postoperative days. It is nonetheless possible that subtle cellular changes, identifiable only by electron microscopy, might be present early after ischemia and might later return to normal. Recovery of normal esophageal motility could then parallel the return of muscle function. The observed cytologic changes in ganglion cells in the early postoperative period might reflect a profound disturbance in cell function. Later, some cells die and others recover to permit restoration of function despite reduction in numbers. It has been said that development of esophageal motor disturbance requires that ganglion cells be reduced below a critical number. Köberle, studying Chagas' disease, puts this at less than 50 per cent of normal. In the present study, ganglion cell damage may not have been severe enough
to cause a permanent motor disturbance. The observed pattern of esophageal motility disturbance with recovery might be explained equally well by a combination of these two possible mechanisms. Whatever the true explanation may be, the techniques employed in this study were successful in damaging ganglion cells and in temporarily altering esophageal motility. Additional work will be required to define the precise interrelations of these objective findings.

Summary

The lower end of the esophagus and the upper part of the stomach were made ischemic for 4 hours in 15 dogs. The esophagus became dilated in the majority of the animals, but eventually reverted to normal size. Definite motility disturbances occurred in the early postoperative period, consisting of absence of peristalsis in the body of the esophagus and failure of the gastroesophageal sphincter to relax, but recovery occurred in the majority. The ganglion cells in Auerbach's plexus were damaged and their numbers eventually reduced.

The precise mechanism responsible for the abnormalities observed in the early period after ischemia remains to be elucidated. It is clear, however, that the lower esophagus and the esophagogastric junction can function normally in the absence of normal numbers of ganglion cells in Auerbach's plexus.

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