

# Epidural migration of new methylene blue in 0.9% sodium chloride solution or 2% mepivacaine solution following injection into the first intercoccygeal space in foal cadavers and anesthetized foals undergoing laparoscopy

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**Objective**—To determine the relationship between epidural cranial migration and injectate volume of an isotonic solution containing dye in laterally recumbent foal cadavers and evaluate the cranial migration and dermatome analgesia of an epidural dye solution during conditions of laparoscopy in foals.

**Animals**—19 foal cadavers and 8 pony foals.

**Procedures**—Foal cadavers received an epidural injection of dye solution (0.05, 0.1, 0.15, or 0.2 mL/kg) containing 1.2 mg of new methylene blue (NMB)/mL of saline (0.9% NaCl) solution. Length of the dye column and number of intervertebral spaces cranial and caudal to the injection site were measured. Anesthetized foals received an epidural injection of dye solution (0.2 mL/kg) containing saline solution or 2% mepivacaine. Foals were placed in a 10° head-down position, and pneumoperitoneum was induced. Dermatome analgesia was determined by use of a described electrical stimulus technique. Foals were euthanized, and length of the dye column was measured.

**Results**—Epidural cranial migration of dye solution in foal cadavers increased with increasing volume injected. No significant difference was found in epidural cranial migration of a dye solution (0.2 mL/kg) between anesthetized foals undergoing conditions of laparoscopy and foal cadavers in lateral recumbency. Further cranial migration of the dye column occurred than indicated by dermatome analgesia.

**Conclusions and Clinical Relevance**—Epidural cranial migration increases with volume of injectate. On the basis of dermatome analgesia, an epidural injection of 2% mepivacaine (0.2 mL/kg) alone provides analgesia up to at least the caudal thoracic dermatome and could permit caudal laparoscopic surgical procedures in foals. (*Am J Vet Res* 2005; 66:1324–1329)

In the first few weeks of life, foals may require surgical intervention for abdominal exploration or repair of a ruptured bladder, patent urachus, or hernia.<sup>1-3</sup> Traditionally, these procedures are performed by use of open surgical techniques<sup>1,2</sup>; however, laparoscopic surgical approaches have recently been described.<sup>3-6</sup> Laparoscopic techniques have several advantages over conventional surgical techniques; they are minimally invasive, associated with a low patient morbidity rate, and allow for rapid postoperative recovery.<sup>7,8</sup> However, they are associated with longer surgical times and potentially negative cardiopulmonary effects, compared with conventional surgical approaches performed under general anesthesia in horses.<sup>9</sup>

In a recently reported large retrospective study,<sup>10</sup> the mortality rate in foals < 1 month of age was higher than in mature horses both during and following general anesthesia. However, to date, only general anesthesia, with inhalant agents or a combination of injectable and inhalant agents, has been described in foals undergoing open or laparoscopic surgery.<sup>11</sup> The greater risk of anesthetic complications in foals may partially be the result of the reduced ability of neonates to adapt to the profound hemodynamic alterations caused by the general anesthetic agents currently available.<sup>10</sup> An anesthetic regimen other than general anesthesia or one that minimizes the use of general anesthetics by incorporating local anesthetic techniques would be a useful addition to minimally invasive surgical techniques, such as laparoscopy, for the management of critically ill equine neonates.

Epidural anesthesia for procedures in the caudal portion of the abdomen has been described in several species and has been shown to decrease the risks associated with general anesthesia in human patients with compromised cardiovascular status.<sup>12,13</sup> In humans,

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epidural anesthesia of the lumbosacral region extending cranially up to the level of the fifth thoracic dermatome is not associated with major cardiovascular alterations; however, when epidural anesthesia extends cranial to the fifth thoracic dermatome, hypotension may occur secondary to sympathetic nerve blockade.<sup>14</sup> The goal, therefore, in performing epidural anesthesia is to provide anesthesia of the necessary abdominal region without the blockade extending cranial to the fifth thoracic dermatome. With this knowledge, outpatient laparoscopy in humans has been found to be a fast, safe examination that can be done at the patient's bedside by use of epidural and local anesthesia.<sup>12,13</sup> In adult horses, goats, calves, and pigs, it has been shown that there is a close relationship between the volume of solution injected into the epidural space and the cranial migration of the solution.<sup>15-18</sup> To the authors' knowledge, there are no reports evaluating the volume of local anesthetic, when injected at the first intercoccygeal space, required to provide adequate cranial abdominal anesthesia for laparoscopy in foals.

The purpose of the study reported here was to determine the relationship between epidural migration and injectate volume of an isotonic solution in foal cadavers placed in lateral recumbency and to evaluate the epidural migration and dermatome analgesia after epidural injection of 2% mepivacaine in anesthetized foals under conditions of laparoscopic surgery. We hypothesized that the epidural cranial migration in foal cadavers placed in lateral recumbency increases with increasing volume injected and that positioning the foals in a 10° head-down position with pneumoperitoneum would influence the degree of epidural cranial migration. We also hypothesized that dermatome analgesia would correlate with the cranial migration of the epidural injectate.

## Materials and Methods

**Part 1 animals**—Nineteen foals that died or were euthanized (pentobarbital sodium, 100 mg/kg, IV) for reasons other than neurologic disease were used within 24 hours of death. They ranged from 0 to 10 days of age (mean, 3.9 days) and included 11 females and 8 males that were either Standardbreds (12 foals) or Thoroughbreds (7). Cadavers weighed 20 to 60.5 kg (mean, 44.1 kg). Foals were randomly assigned to 1 of 4 treatment groups on the basis of the volume of an isotonic dye solution injected into the epidural space by use of a catheter placed in the first intercoccygeal space. The epidural injectate was composed of saline (0.9% NaCl) solution in which new methylene blue (NMB) was diluted at a concentration of 1.2 mg of NMB/mL of saline solution, as previously described.<sup>16-18</sup> The epidural injectate volume was 0.05 mL/kg for group 1 (n = 5), 0.1 mL/kg for group 2 (5), 0.15 mL/kg for group 3 (4), and 0.2 mL/kg for group 4 (5). All epidural injections were performed by the same researcher (JLL) with the foal placed in left lateral recumbency.

**Part 1 epidural injections and measurements**—Hair was clipped from the area over the sacrococcygeal region. A commercially available nylon 19-gauge epidural catheter<sup>a</sup> was placed through a 17-gauge Tuohy needle inserted at the first intercoccygeal space by use of a previously described technique.<sup>19</sup> To optimize the consistency of the location of the distal tip of the catheter, the distance from the first intercoc-

cygeal space and the sacrococcygeal junction was measured. On the basis of the premeasured value, the catheter was then advanced past the tip of the Tuohy needle to the sacrococcygeal junction. After successful placement of the epidural catheter, the previously determined volume of dye solution was injected over a period of 3 minutes. Forty minutes following the injection, the vertebral columns were isolated and sagittally sectioned by use of a band saw. The total vertebral column length of each foal was measured from the occipital condyle to the second coccygeal vertebra. For each foal, the dye column length was measured in centimeters, and the number of intervertebral spaces traveled by the dye was counted from the first intercoccygeal space to the most cranial location of the dye column. The cranial extent of the dye was thus expressed in centimeters, number of intervertebral spaces, and actual anatomic location between 2 vertebral bodies for each foal. If the dye extended past the midline of a vertebra, the space cranial to that vertebra was counted. The length of the dye column caudal to the injection site was also measured. It has been shown that variations in the number of vertebrae exist in the lumbosacral area of the horse<sup>20</sup>; therefore, the total number and type of vertebrae in each foal were counted during postmortem examination.

**Part 2 animals**—Eight healthy pony foals were used in this portion of the study. They weighed between 40 and 75 kg (mean, 52.4 kg) with 3 females and 5 males aged between 34 to 39 days (mean, 36 days). Foals were assessed as healthy on the basis of physical examination findings, CBC determination, and results of serum biochemical analysis. They were part of a larger terminal laparoscopic research project.<sup>21</sup> All procedures were approved by the Animal Care Committee of the University of Guelph.

**Part 2 procedures**—Anesthesia was induced by use of a combination of xylazine hydrochloride (0.8 mg/kg, IV) and ketamine hydrochloride (2.2 mg/kg, IV). Foals were intubated, and general anesthesia was maintained with isoflurane in oxygen delivered with a circle breathing system. A catheter (8-F polyvinyl chloride feeding tube<sup>b</sup>) was placed in the lumen of the orotracheal tube so that its distal tip lay level with the tip of the tube. The proximal end of the catheter was connected to an infrared absorption spectrophotometer<sup>c</sup> to measure end-tidal gas concentrations. Prior to each experimental period, the spectrophotometer was calibrated with manufacturer-recommended gases containing known concentrations of isoflurane.<sup>d</sup> Intermittent positive-pressure ventilation was maintained throughout the anesthetic period, with the tidal volume set at 10 to 15 mL/kg and the rate adjusted to maintain end-tidal carbon dioxide concentrations between 35 and 45 mm Hg. The end-tidal isoflurane concentration was maintained at 1.4%. Foals were placed in left lateral recumbency, and an epidural catheter was placed by use of the same technique and commercially available epidural catheters as described in part 1. Foals were then randomly assigned to a control (n = 4) or treatment (4) group. In the control group, a dye solution (0.2 mL/kg) consisting of diluted NMB in saline solution (1.2 mg of NMB/mL of saline solution) was injected in the epidural space via the epidural catheter by use of the same technique as in part 1. In the treatment group, a dye solution (0.2 mL/kg) consisting of NMB diluted in 2% mepivacaine solution (1.2 mg of NMB/mL of 2% mepivacaine solution) was injected in the epidural space via the epidural catheter by use of the same technique as in part 1.

Following completion of the epidural injection, foals were positioned in dorsal recumbency. Twenty minutes later, each foal was tilted to a 10° head-down position. By use of a high-flow carbon dioxide insufflator, the abdomen was then insufflated to a pressure of 12 mm Hg through a laparoscopic

cannula placed in the cranial abdomen. Forty-five minutes following the epidural injection, foals were returned to a horizontal position and their abdomens manually deflated through the open laparoscopic cannula. Sixty minutes after the epidural injection, the end-tidal isoflurane was reduced to 0.9% and an electrical stimulus<sup>6</sup> was used to assess dermatome analgesia, as previously described.<sup>22</sup> Sixty minutes was chosen as it was speculated that the mepivacaine would still be effective on the basis of its known duration of action of 90 to 120 minutes.<sup>23</sup> A positive response to the stimulus was regarded as purposeful movement of the head or limbs following electrical stimulation. Initial stimulation was performed on a cranial location that was anticipated to be far from the most cranial extent of the analgesia to ensure that the foals would respond. This area was chosen in the cervical region at the approximate location of the ventral branch of the sixth cervical vertebra.<sup>19</sup> Subsequent to confirming purposeful response in each foal, bilateral, sequential stimulation in a right then left caudocranial direction was performed. The areas stimulated included the caudal lumbar, cranial lumbar, caudal thoracic, middle thoracic, and cranial thoracic dermatomes as previously described.<sup>19</sup> These dermatomes represented areas of the skin innervated by the spinal nerves located between S2 and L6, between L6 and L1, thoracic nerve 16, thoracic nerve 12, and thoracic nerve 8,<sup>19</sup> respectively. If no purposeful movement was observed when the cranial thoracic dermatome was tested, electrical stimulation was moved cranially in 0.5-cm increments until movement was detected. The electrodes of the stimulator were attached to towel clamps that were placed into the subcutaneous tissues, 5 cm apart, over the location of the dermatome to be tested. The stimulator was initially set to deliver 50 V at a frequency of 1 Hz every 10 milliseconds. The voltage was increased incrementally every 3 seconds up to 80 V over a period of 15 seconds or until a positive response was obtained as assessed by an individual blinded to the treatment. Foals were euthanized (pentobarbital sodium, 100 mg/kg, IV) while under general anesthesia, and their vertebral columns were sectioned. The vertebral column and dye column were measured as in part 1. Position of the epidural catheter was recorded for each foal at the time of postmortem examination.

**Statistical analysis**—Dye length-to-vertebral column length ratio and the number of intervertebral spaces traveled by the dye solution were tested by use of an ANOVA for part 1 of the study. If the overall F test was significant, a post hoc Tukey test was applied to identify significant differences among groups. A Shapiro-Wilk test was used to determine whether the residuals were normally distributed.

In part 2 of the study, a Student *t* test was applied to determine whether a significant difference existed in the mean ratio of dye length to vertebral column length and in the number of intervertebral spaces between control and treatment groups and between live and dead foals (from part 1) receiving an epidural injection volume of 0.2 mL/kg. The number of intervertebral spaces traveled cranially by the dye column on postmortem examination was compared with the anatomic cranial extent of the dermatome analgesia by use of a Fischer exact test. A Shapiro-Wilk test was used to determine whether the residuals were normally distributed. For all tests, values of *P* < 0.05 were considered significant.

## Results

**Part 1**—All foals had 7 cervical, 18 thoracic, 6 lumbar, and 5 sacral vertebrae. All catheters were within the epidural space, and the dye solution was easily observed to have stained the epidural fat and dura mater in all foals. On gross examination, the dye appeared to have migrated an equal distance on the left and right of the epidural space in all cases. Caudal migration of the dye was less than a half of a coccygeal vertebra in each foal.

The dye length-to-vertebral column length ratio and the number of intervertebral spaces traveled by the dye solution increased with increasing volume injected and were significantly different among groups. Specifically, these variables were significantly greater in group 4, compared with groups 1 through 3, and significantly less in group 1, compared with groups 2 through 4 (Table 1). No significant differences were found between groups 2 and 3 in either measured variable.

**Part 2**—No complications developed in any foal. All foals responded to electrical stimulation of the cervical region, and all foals in the control group responded to electrical stimulation at all sites. In the treatment group, a positive response was observed to electrical stimulation immediately cranial to the caudal thoracic dermatome in 2 foals, immediately cranial to the middle thoracic dermatome in 1 foal, and immediately (< 0.5 cm) cranial to the cranial thoracic dermatome in 1 foal. In 3 foals, the positive response to stimulation was caudal to the most cranial portal site used in 2

Table 1—Cranial migration of 4 different volumes of a dye solution injected at the first intercoccygeal space in foal cadavers.

Group (volume)	Mean foal weight (kg [range])	Dye length-to-vertebral column length ratio			No. of IVS traveled by dye			Cranial location of dye (range)		
		Mean (range)	Group 1*	Group 2*	Group 3*	Mean (range)	Group 1*		Group 2*	Group 3*
Group 1 (0.05 mL/kg)	66.7 (46–118)	0.21 (0.19–0.23)	—	—	—	9 (7–11)	—	—	—	L3-L4 (L1-L2 to L5-L6)
Group 2 (0.1 mL/kg)	60.2 (41.5–118)	0.31 (0.25–0.4)	0.046†	—	—	14.8 (13–19)	0.009†	—	—	T15-T16 (T11-T12 to T17-T18)
Group 3 (0.15 mL/kg)	36.25 (20–42)	0.34 (0.28–0.37)	0.020†	0.602	—	14.75 (12–16)	0.014†	0.981	—	T15-T16 (T14-T15 to T18-L1)
Group 4 (0.2 mL/kg)	37.6 (21.5–47.5)	0.56 (0.38–0.67)	< 0.001†	< 0.001†	< 0.001†	25.8 (22–31)	< 0.001†	< 0.001†	< 0.001†	T5-T6 (C7-T1 to T8-T9)

\**P* values between groups. †Significant (*P* < 0.05) value between groups. IVS = Intervertebral spaces.

Table 2—Cranial migration of an epidural injection of 0.9% NaCl (control group) or 2% mepivacaine (treatment group) in anesthetized foals placed in dorsal recumbency for laparoscopic surgery.

Group	Mean values (range)			
	Foal weight (kg)	Dye length-to-vertebral column length ratio	No. of IVS traveled by dye	Cranial location of dye (range)
Control (0.2 mL/kg without 2% mepivacaine)	54.9 (47–75)	0.53 (0.43–0.60)	23.75 (21–28)	T6-T7 (T2-T3 to T9-T10)
Treatment (0.2 mL/kg with 2% mepivacaine)	50 (40.5–63.5)	0.54 (0.43–0.61)	23.25 (20–25)	T7-T8 (T5-T6 to T10-T11)
<i>P</i> values*	ND	<i>P</i> = 0.95	<i>P</i> = 0.98	NA

ND = Not determined. NA = Not applicable.  
See Table 1 for remainder of key.

published studies<sup>5,21</sup> on laparoscopy in foals.

All catheters were determined to be within the epidural space on postmortem examination, and all foals had 7 cervical, 18 thoracic, 6 lumbar, and 5 sacral vertebrae. The dye was easily observed to have stained the epidural fat and dura mater in all foals, and on gross examination, the dye appeared to have migrated an equal distance on the left and right of the epidural space in all cases. Caudal migration of the dye was less than a half of a coccygeal vertebra in each foal. The dye traveled a mean of 49.6 cm (range, 38 to 56 cm) in the control group and 47.8 cm (range, 40 to 55.5 cm) in the treatment group. The mean location of dye migration in the control group was between thoracic vertebrae 6 and 7 and in the treatment group was between thoracic vertebrae 7 and 8 (Table 2). In all foals in the treatment group, the dye column migrated further cranial than indicated by dermatome analgesia determined by use of the electrostimulator. No significant differences were found in the dye length-to-vertebral column length ratio or in the number of intervertebral spaces between the 2 groups of foals. No significant difference was found in the distance of cranial epidural migration between cadavers receiving epidural injectate (0.2 mL/kg) and anesthetized foals undergoing laparoscopic positioning with an epidural injectate of 0.2 mL/kg (ie, dye length-to-vertebral column length ratio, *P* = 0.336; number of intervertebral spaces, *P* = 0.243).

## Discussion

In our study, the cranial migration of an isotonic solution injected into the epidural space in euthanized foals placed in lateral recumbency increased with increasing volume injected, as observed in other large animal species.<sup>15-18</sup> In addition, cranial migration of the epidural injectate was not influenced by conditions of laparoscopy or whether the foals were anesthetized or dead at the time of injection. It was hypothesized that gravity from a foal being in a head-down position would cause injectate to spread further cranially than in a foal in a horizontal and lateral position. On the basis of the large volume of injectate, this was of particular importance because of the potential for blockade cranial to thoracic dermatome 5 and its resulting cardiovascular consequences.<sup>15</sup> Gravity produced by the sitting position has been shown to reduce cranial spread of epidural anesthesia in obese humans but not in lean patients.<sup>24</sup> Because foals are very lean patients,

this may explain why no increase in cranial spread was seen with the head-down position versus the lateral position. Results of another study<sup>25</sup> in humans reveal that the cranial movement of epidural solution is only minimally affected by gravity. It is possible that a 10° head-down position did not provide a steep enough angle to cause gravitational effects in the foals of our study. Since a 10° head-down position is clinically steep enough to perform laparoscopic surgery in foals, it is likely that gravity can be eliminated as an important factor when performing epidural anesthesia for laparoscopic surgery in this population. Several operator-controlled factors also influence the migration of solutions injected into the epidural space, including the volume of injectate, site and speed of injection, concentration of anesthetic solution, and direction of bevel.<sup>15</sup> The effect of volume of injectate on the cranial migration of solution was evaluated in our study as it has consistently been shown to influence the cranial migration of an epidural injectate in several species.<sup>15-18</sup> Furthermore, it is a variable easily adjusted by a veterinarian to achieve a desired distribution of injectate in the epidural space.<sup>15-18</sup> A variety of drugs have been used for epidural injection in large animals, including local anesthetics such as 2% lidocaine,  $\alpha$ -2 adrenergic agonists such as xylazine, and opioids such as morphine.<sup>19</sup> Several different volumes of epidural injectate have also been used in large animals. In cattle, epidural injectate volumes of 0.2 to 0.5 mL/kg administered at the first intercoccygeal space have been used to achieve blockade of spinal nerves originating from the 13th thoracic to the 3rd lumbar vertebrae, providing effective anesthesia for procedures in the caudal portion of the abdomen.<sup>15</sup> In adult horses, epidural injection of volumes of 0.022 and 0.11 mL/kg have been reported<sup>16</sup> to result in a cranial migration up to 6 and 10 intervertebral spaces, respectively. It was postulated that the most cranial portal site used in laparoscopic procedures in foals is located at approximately the level of the eighth thoracic dermatome or the eighth thoracic spinal nerve. On the basis of this, epidural injectate volumes up to 0.2 mL/kg were evaluated in part 1 of our study.

On the basis of the distribution of dye solution in the epidural space in part 1 of our study, it was anticipated that a volume of 0.2 mL of 1.2 mg of NMB/mL mixed with 2% mepivacaine/kg (part 2 of our study)

would result in adequate distribution of the agent to provide analgesia in regions supplied by dermatomes caudal to the eighth thoracic spinal nerve. In a study<sup>26</sup> performed in goats with 0.75% bupivacaine and 2% lidocaine with epinephrine at 0.25 and 0.2 mL/kg, respectively, adequate analgesia for laparotomy was provided in 13 of 17 goats and 5 of 7 goats, respectively. In another study,<sup>27</sup> 2% lidocaine (0.18 to 0.24 mL/kg) with xylazine (0.05 mg/kg) was injected epidurally in calves and was found to provide adequate analgesia for umbilical resection, only if additional local infiltration was provided cranial to the umbilicus. For our study, to determine the actual analgesia provided by the chosen epidural volume (0.2 mL/kg), we did not provide local infiltration of the portal sites. Although laparoscopy is less invasive than laparotomy, we do anticipate the need for local infiltration in clinical situations. To reduce the effect of other factors that influence the distribution of drugs in the epidural space, the concentration of injectate was maintained in both parts of our study, all injections were made at the same site by use of an epidural catheter, and the speed of injection was consistent over 3 minutes.

The cranial extent of sensory blockade associated with epidural administration of mepivacaine was not as far cranial as the dye solution indicated on postmortem examination. Previous investigators<sup>15,28</sup> have had similar findings when evaluating epidural dye solutions and dermatome analgesia. Burn et al<sup>28</sup> showed that the physical spread of dye and the neuraxial spread tested by electrostimulation were different, thus concluding that the physical spread alone was not responsible for the extent of neural block. It has also been shown that drugs with different pharmacokinetics will cause different desensitization patterns with similar fluid volumes.<sup>16</sup> The pharmacodynamic and pharmacokinetic differences between the dye and the local anesthetic solutions used could play a role. For example, the concentration of local anesthetic required to result in blockade, compared with the concentration of dye to result in epidural tissue staining, may not have been equivalent. Similarly, the distribution of the local anesthetic could have been different than the dye in the epidural tissues. New methylene blue was chosen for our study as it is a commonly used dye to mark tissue.<sup>19</sup> The concentration of NMB in saline solution (1.2 mg/mL) used in our study was chosen on the basis of the results of previous studies,<sup>15-18</sup> which found that this concentration remained stable at cool temperatures, stained the epidural fat and dura mater well, and did not increase the viscosity or specific gravity of the solution beyond the normal CSF. New methylene blue dye determines the absolute spread in the epidural space and not diffusion through the meninges or CSF<sup>16</sup>; however, this may not represent the distribution of a local anesthetic such as mepivacaine. Thus, this could also explain the discrepancy between epidural dye spread and dermatome analgesia found in foals in our study. Despite these limitations, the NMB dye solution provided valuable information with respect to the volume of injectate and the distribution within the epidural space in foals.

In our study, mepivacaine was evaluated on the basis of its 90- to 120-minute duration of effect.<sup>23</sup> This

duration is adequate for most laparoscopic procedures in foals and would not result in prolonged recumbency postoperatively. Combinations of analgesic agents with local anesthetics, such as opioids or  $\alpha$ -2 adrenergic receptor agonists with local anesthetics, are commonly used for epidural anesthesia in large animal patients.<sup>19</sup> Further study is warranted to determine the combination of agents that will result in the optimal quality and duration of epidural anesthesia in foals.

The results of our study provide clinicians with a guide to epidural injectate volume when a specific distribution of epidural anesthesia is desired. An epidural injection of an isotonic solution (0.2 mL/kg) provided analgesia consistently to at least the caudal thoracic dermatome in foals. Caudal thoracoscopic procedures could be performed with this technique; however, local infiltration would be necessary for more cranial portal sites. Studies evaluating volumes in excess of 0.2 mL/kg in addition to local infiltration of portal sites should be performed before this technique is used clinically.

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- a. Benlan Inc, Oakville, ON, Canada.
  - b. Bard Canada Inc, Mississauga, ON, Canada.
  - c. Criticare 1100 patient monitor, Criticare Systems, Waukesha, Wis.
  - d. Scott Medical Products, Plumsteadville, Pa.
  - e. Grass SD9 stimulator, Grass Medical Instruments, Quincy, Mass.
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