

# Validation of several types of noxious stimuli for use in determining the minimum alveolar concentration for inhalation anesthetics in dogs and rabbits

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**Objective**—To compare 3 types of noxious stimuli applied to various anatomic areas of anesthetized dogs and rabbits for determination of the minimum alveolar concentration (MAC).

**Animals**—10 dogs and 10 rabbits.

**Procedure**—Dogs were anesthetized with isoflurane and halothane in a randomized order. Rabbits were anesthetized with isoflurane. The MAC was determined by skin incision on the lateral aspect of the chest; clamping of the tail, paw of the forelimb, and paw of the hind limb; and application of electrical current to the oral mucosa (dogs only), forelimb, and hind limb. The MAC was the end-tidal concentration midway between the value permitting and preventing purposeful movement in response to noxious stimuli.

**Results**—In dogs, mean  $\pm$  SEM MAC for isoflurane was  $1.27 \pm 0.05\%$  for clamping stimuli,  $1.36 \pm 0.04\%$  for oral electrical stimulation,  $1.35 \pm 0.04\%$  for electrical stimulation to the limbs, and  $1.01 \pm 0.07\%$  for surgical incision. The MAC for halothane was  $0.97 \pm 0.03\%$  for tail clamping,  $0.96 \pm 0.03\%$  for clamping of the limbs,  $1.04 \pm 0.03\%$  for electrical stimulation, and  $0.75 \pm 0.06\%$  for surgical incision. In rabbits, MAC for isoflurane was  $2.08 \pm 0.02\%$  for clamping stimuli,  $2.04 \pm 0.02\%$  for electrical stimulation, and  $0.90 \pm 0.02\%$  for surgical incision. The MAC for surgical incision was significantly lower than values for the other methods in both species.

**Conclusions and Clinical Relevance**—Use of electrical current and clamping techniques resulted in similar MAC values. Surgical incision underestimated MAC values in dogs and rabbits. (*Am J Vet Res* 2003;64:957–962)

The minimum alveolar concentration (MAC) is the alveolar concentration of an inhalation anesthetic that prevents movement in 50% of subjects in response to a noxious stimulus. It is the standard method for comparing potency of inhalation anesthetics and also enables researchers to study the effects of various parenterally administered anesthetic drugs on inhalation anesthetic requirements.

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A commonly used supramaximal noxious stimulus for MAC determinations in animals is clamping the tail with a hemostat. The tail is clamped continuously for the duration of the stimulation (30 to 60 seconds) or until a gross purposeful movement is evident (jerking or twisting motion of the head or running motion of the extremities).<sup>1,3</sup> Another method is the use of electrical stimulation (30 to 50 V at 50 cycles/s for 10 milliseconds through needles placed in the cheek, limbs, or sensitive mucosa).<sup>2,5</sup>

In humans, skin incision is the method of choice for determination of MAC, although electrical stimulation has also been used.<sup>3</sup> In contrast, skin incision was described as a nonsupramaximal stimulus in dogs anesthetized with halothane, which resulted in lower MAC values.<sup>2</sup> That study involved the use of only 3 dogs, and additional studies have not been conducted to validate this statement or determine whether it is true for other domestic species.

Most MAC determinations in animals have been performed by use of 1 of the modalities of noxious stimulus applied only to 1 anatomic area. Other than a study<sup>2</sup> conducted in 1965, none of the studies have compared multiple types of noxious stimuli applied to various anatomic areas.

The MAC values reported in various studies differ even for the same species, in part because of the lack of uniformity in the type of noxious stimulus used as well as the anatomic area to which it was applied. It has been documented<sup>2,6,7</sup> that MAC may vary between anatomic sites when the same modality of noxious stimulus is applied. In addition, there is subjectivity in the assessment of purposeful movement and description of the mode of application of the noxious stimulus among investigators. Use of a clamp has been described as a stimulus applied for 30 to 60 seconds (or a shorter duration if the animal responds before then) by use of various types of hemostats with or without plastic tubing on the hemostats' jaws and applied at full ratchet or only at the first ratchet lock<sup>2,3,8</sup>; electrical stimulation has been applied for up to 60 seconds or as a single stimulus for a few seconds.<sup>1,2,4,9</sup>

The purpose of the study reported here was to determine MAC values for isoflurane and halothane by use of various noxious stimuli applied to several anatomic areas in 2 species, in accordance with a standardized protocol. This would enable us to validate results obtained by Eger et al<sup>2</sup> for halothane in dogs and determine whether those results are consistent

with results for the use of isoflurane in dogs and rabbits.

## Materials and Methods

**Animals**—Ten healthy mixed-breed dogs (4 females and 6 males) that weighed 18.1 to 23.6 kg and 10 New Zealand White rabbits (7 females and 3 males) that weighed 3.2 to 4.1 kg were used in the study. Dogs were anesthetized twice (once with halothane and once with isoflurane) in a random order, and rabbits were anesthetized once with isoflurane. Dogs were allowed to recover from anesthesia at the end of the study, whereas rabbits were euthanized after completion of MAC determinations. The study was approved by the Institutional Animal Care and Use Committee of the University of Florida.

**Insertion of instruments**—Anesthesia was induced in dogs and rabbits with inhalation agents in oxygen by use of a face mask. The trachea was then intubated, and anesthesia was maintained by administration of the inhalant in oxygen by use of a coaxial circuit system (ie, Bain breathing circuit) with an oxygen flow of 3 to 4 L/min in dogs and 2 L/min in rabbits.

Instruments were inserted during a 30-minute anesthetic equilibration period. End-tidal concentrations of inhalation anesthetic and CO<sub>2</sub> were monitored by use of an infrared gas analyzer<sup>a</sup> calibrated before each experiment by use of a standardized calibration gas mixture<sup>b</sup> designed for the analyzer. Mechanical ventilation was used, and the end-tidal CO<sub>2</sub> concentration was maintained between 30 and 40 mm Hg. In a few animals, arterial blood samples were collected and used to verify that PaCO<sub>2</sub> was within the reference range. Esophageal temperature was monitored electronically and maintained between 37.0 and 39°C. The ECG, heart rate, and indirect blood pressure<sup>c</sup> were monitored throughout each experiment. A catheter was inserted in a cephalic vein, and lactated Ringer's solution was administered at a rate of 3 mL/kg/h.

**Noxious stimuli**—Determinations of the MAC for isoflurane were conducted by use of established techniques.<sup>2</sup> Three modalities of noxious stimuli were applied (skin incision on the lateral costal area; clamping of the tail, paw of a forelimb, and paw of a hind limb; and electrical current applied to the oral mucosa [dogs only], forelimb, and hind limb). Order of application of the modality and anatomic location was randomized, except that the skin incision was always performed first and only in the first 2 consecutive measurements.

The first skin incision was made in the region of the last rib, and the second skin incision was made in the region of the ninth rib to avoid damage of the nerves supplying each surgical area. Skin incisions were made by use of the up-down method for sequential sampling of quantal-response data.<sup>10</sup> This technique uses the result of each incision to determine the concentration sampled on the subsequent incision. For the study reported here, each skin incision was made at different end-tidal concentrations. When the dog or rabbit did or did not respond with gross purposeful movement to the first skin incision, the end-tidal concentration was increased or decreased, respectively, by 0.1% prior to the second incision. Purposeful movement was defined as a jerking or twisting motion of the head or running motion of the extremities.<sup>3</sup> When gross purposeful movement was detected for both skin incisions, the end-tidal anesthetic concentration was increased by 0.1% for the subsequent animal. Conversely, when gross purposeful movement was not detected for either skin incision, the anesthetic concentration was decreased by 0.1% for the subsequent animal. Skin incisions in dogs were sutured by use of 3-0 prolene,<sup>c</sup> and suture material was removed 7 days later.

Clamping of the tail was performed from distal to proximal, starting approximately 10 cm from the base of the tail in dogs and 5 cm from the base of the tail in rabbits and proceeding proximally on subsequent determinations. Each paw was clamped only by including the third and fourth digits. The clamping technique was performed by use of a 24-cm sponge forceps<sup>d</sup> with protective plastic tubing on each jaw that was clamped to the first notch until gross purposeful movement was detected or a period of 60 seconds elapsed.

Electrical current<sup>e</sup> consisted of 50 V at 50 cycles/s for 10 milliseconds applied SC by inserting two 25-gauge, 1-inch needles 5 cm apart in the region of the ulna in the forelimb, region of the tibia in the hind limb, and mandibular mucosa underneath the canine and first molar in the oral cavity (dogs only). The protocol consisted of applying 2 single stimuli followed by 2 continuous stimuli applied for 2 to 3 seconds, with 5-second intervals between all 4 stimuli. When gross purposeful movement was elicited before the cycle was completed, the electrical stimulus was discontinued immediately.

After an initial equilibration period of at least 30 minutes at an end-tidal concentration of 1.4% halothane or 1.7% isoflurane in dogs and 2% isoflurane in rabbits, the end-tidal concentration was decreased to 0.85% for halothane and 1% for isoflurane in the first dog of each group and to 1% isoflurane in the first rabbit, and it was maintained at this concentration for at least 20 minutes. Noxious stimuli were applied, and evidence of, or lack of, gross purposeful movement was determined. Then, the end-tidal anesthetic concentration was increased (or decreased) by 0.1% and maintained constant for at least 20 minutes, and the noxious stimuli procedure was repeated. One to 2 minutes were allowed between stimuli when a positive response was elicited, but no time was allowed between stimuli when a negative response was detected. This process was continued until purposeful movement ceased (increases in anesthetic concentration) or returned (decreases in anesthetic concentration). The end-tidal anesthetic concentration midway between the highest value at which we detected purposeful movement and the lowest value that prevented purposeful movement was recorded as the MAC value for the inhalation anesthetic for each noxious modality for that animal.

**Statistical analysis**—Data for the clamping and electrical modalities in each group of animals were analyzed by use of repeated-measures ANOVA. Time of MAC determination was determined for both species and compared in dogs for isoflurane and halothane by use of a paired Student *t* test.

The MAC values for skin incisions were calculated by use of 2 separate methods.<sup>3</sup> These simplified clinical analyses yield values that are equal to those produced by a more rigorous examination of quantal responses.<sup>3,11</sup> The first method used a curve-fitting technique to estimate MAC, whereas the second method used mathematical averaging. Ideally, values calculated by use of each method should be approximately equal.

For the MAC determination by use of the curve-fitting technique, the response to surgical stimulus was recorded as detected or not detected for a given alveolar concentration of inhalation anesthetic. The percentage of animals that had purposeful movement while being administered a given concentration of inhalation anesthetic was calculated and plotted against the anesthetic concentration. A 3-parameter best-fit line<sup>e</sup> was fitted in accordance with the following equation:

$$y = \frac{a}{1 + e^{\frac{-(x-x_0)}{b}}}$$

where *y* is a given response, *a* is the maximal response, *b* is the minimal response, *x* is the anesthetic concentration for a

Table 1—Mean  $\pm$  SEM minimum alveolar concentration (MAC) values determined for halothane and isoflurane in 10 dogs and isoflurane in 10 rabbits

Anesthetic	Clamping			Electrical stimulation		
	Tail	Forelimb	Hind limb	Oral mucosa	Forelimb	Hind limb
Halothane Dogs	0.97 $\pm$ 0.03	0.96 $\pm$ 0.03	0.96 $\pm$ 0.03	1.04 $\pm$ 0.03	1.04 $\pm$ 0.03	1.04 $\pm$ 0.03
Isoflurane Dogs	1.27 $\pm$ 0.05	1.27 $\pm$ 0.05	1.27 $\pm$ 0.05	1.36 $\pm$ 0.04	1.35 $\pm$ 0.04	1.35 $\pm$ 0.04
Rabbits	2.08 $\pm$ 0.02	2.08 $\pm$ 0.02	2.08 $\pm$ 0.02	ND	2.04 $\pm$ 0.02	2.04 $\pm$ 0.02

ND = Not determined.

given response, and  $x_0$  is the anesthetic concentration at 50% response (ie, MAC).

The MAC determination was also calculated by use of mathematical averaging. When gross purposeful movement was observed at a concentration of inhalant anesthetic and no gross purposeful movement was observed at the subsequent concentration of inhalant anesthetic, the MAC value for that animal was considered to be the simple mathematical average of the 2 concentrations. Mean  $\pm$  SEM of these average MAC values for each animal was calculated. Data for animals that had only a single response for the various anesthetic concentrations were excluded from this analysis. Thus, some animals considered in the analysis for the curve-fitting technique were excluded for the mathematical-averaging technique.

The MAC values were reported as mean  $\pm$  SEM. Time of MAC determination was reported as mean  $\pm$  SD. Values of  $P < 0.05$  were considered significant.

## Results

The MAC values for halothane and isoflurane were determined (Tables 1 and 2). The MAC of isoflurane and halothane for the skin incision technique was significantly lower than values determined by use of the other methods. In dogs, the MAC of isoflurane was 1.27  $\pm$  0.05% for all clamping stimuli, 1.36  $\pm$  0.04% for electrical current applied to the oral mucosa, 1.35  $\pm$  0.04% for electrical current applied to the forelimb and hind limb, and 1.01  $\pm$  0.07% for the skin incision. The MAC of halothane was 0.97  $\pm$  0.03% for tail clamping, 0.96  $\pm$  0.03% for clamping applied to paws of the forelimb and hind limb, 1.04  $\pm$  0.03% for all electrical stimuli, and 0.75  $\pm$  0.06% for the skin incision.

In rabbits, the MAC of isoflurane was 2.08  $\pm$  0.02% for all clamping stimuli, 2.04  $\pm$  0.02% for electrical stimulation of the forelimb and hind limb, and 0.90  $\pm$  0.02% for the skin incision.

For MAC values determined by use of a skin incision, quantal and percentage responses were plotted, and curves were fit to the plotted data (Fig 1 to 3).

Table 2—Mean  $\pm$  SEM MAC values determined by use of a skin incision for halothane and isoflurane in dogs and isoflurane in rabbits for each of 2 methods of analysis

Anesthetic	Curve-fitting method		Mathematical average	
	MAC (%)	n	MAC (%)	n
Halothane Dogs	0.75 $\pm$ 0.06	10	0.78 $\pm$ 0.05	6
Isoflurane Dogs	1.01 $\pm$ 0.07	10	1.03 $\pm$ 0.04	6
Rabbits	0.90 $\pm$ 0.02	10	0.90 $\pm$ 0.06	4

Overall, fit of the curves was good ( $R^2$ , 0.95 to 0.99). There was a difference in the total number of observations between the 2 methods as a result of the fact that some animals had only a single response for 2 anesthetic concentrations, which therefore precluded calculation of MAC for that animal for inclusion in the summary data.

Mean  $\pm$  SD time of MAC determinations in dogs was 164  $\pm$  32.4 minutes for halothane and 164  $\pm$  33.7 minutes for isoflurane; these values did not differ significantly. Mean time of MAC determination for isoflurane in rabbits was 155  $\pm$  29.9 minutes.

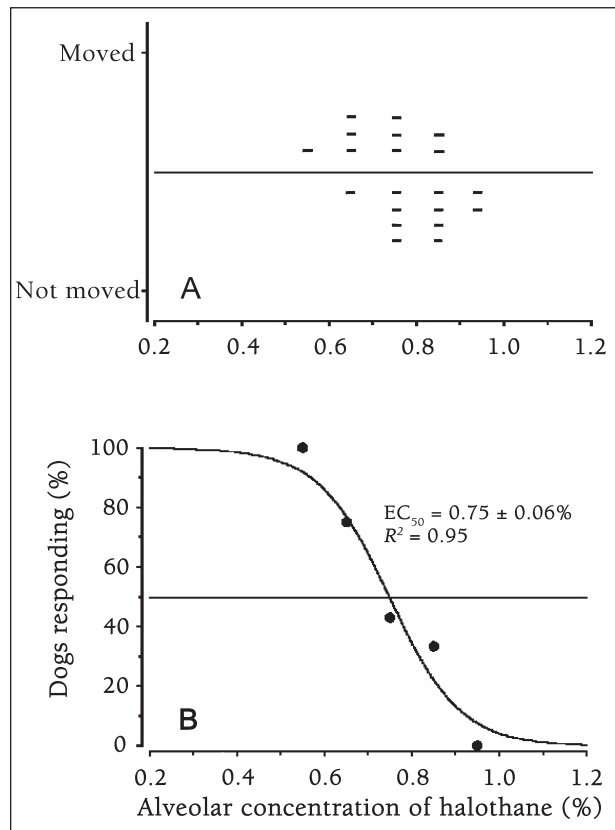


Figure 1—Determination of minimum alveolar concentration (MAC) for halothane in 10 dogs. Response (purposeful movement [Moved] or no purposeful movement [Not moved]) for each dog at various alveolar concentrations of halothane during 2 separate anesthetic administrations was plotted (A), and the percentage of dogs responding was plotted against the alveolar concentration of halothane in accordance with a curve-fitting equation (B). Mean  $\pm$  SEM value for the median effective dose ( $EC_{50}$ , equivalent to the MAC) and the  $R^2$  value were calculated. Horizontal line denotes the value that is 50% of the maximal response.

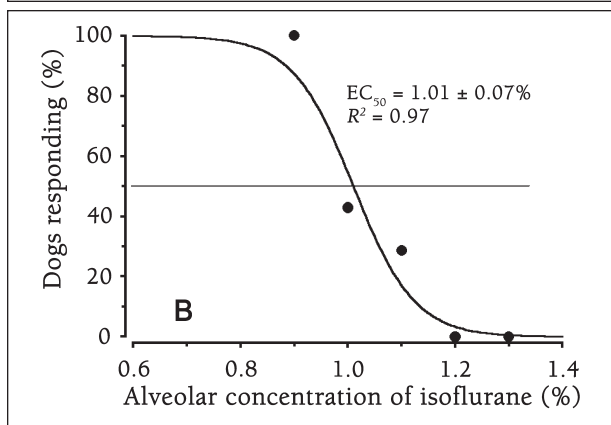
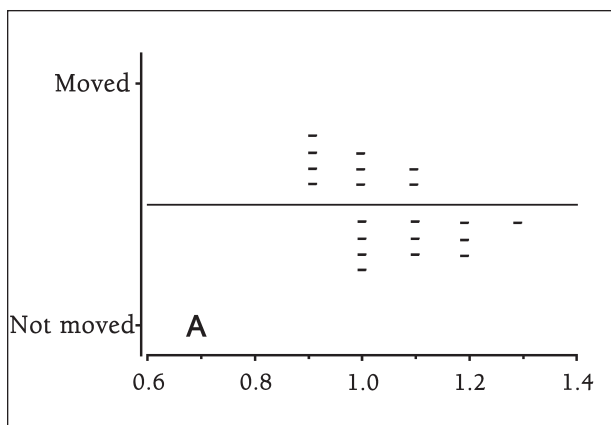


Figure 2—Determination of MAC for isoflurane in 10 dogs. Response (purposeful movement [Moved] or no purposeful movement [Not moved]) for each dog at various alveolar concentrations of isoflurane during 2 separate anesthetic administrations was plotted (A), and the percentage of dogs responding was plotted against the alveolar concentration of isoflurane in accordance with a curve-fitting equation (B). Mean  $\pm$  SEM value for the  $EC_{50}$  (ie, MAC) and the  $R^2$  value were calculated. See Figure 1 for remainder of key.

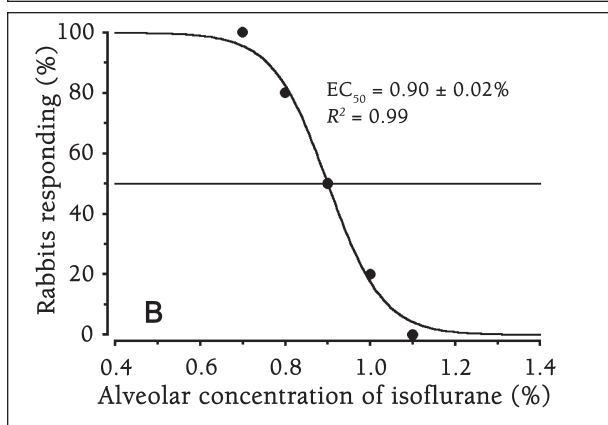
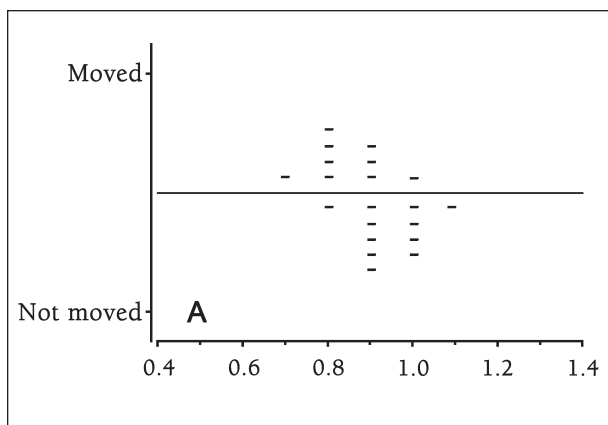


Figure 3—Determination of MAC for isoflurane in 10 rabbits. Response (purposeful movement [Moved] or no purposeful movement [Not moved]) for each rabbit at various alveolar concentrations of isoflurane during anesthesia was plotted (A), and the percentage of rabbits responding was plotted against the alveolar concentration of isoflurane in accordance with a curve-fitting equation (B). Mean  $\pm$  SEM value for the  $EC_{50}$  (ie, MAC) and the  $R^2$  value were calculated. See Figure 1 for remainder of key.

## Discussion

Values of MAC reported in the literature for a single inhalation anesthetic can differ substantially among animals of the same species. For example, the MAC of halothane determined in rabbits by use of the tail-clamping technique has been reported as 0.82,<sup>12</sup> 1.39,<sup>8</sup> and 1.42%,<sup>13</sup> which represents variability of up to 42% between the lowest and highest reported values. The MAC of halothane determined in dogs by use of the tail-clamping technique has been reported as 0.81,<sup>2</sup> 0.93,<sup>14</sup> and 1.03%.<sup>15</sup> Furthermore, the MAC determined in dogs by use of electrical stimulation was 1.04%.<sup>4</sup> Thus, these results in dogs represent variability of up to 22%.

The MAC of isoflurane determined by use of the tail-clamping technique in rabbits has been reported as 2.05% in 1 study<sup>8</sup> and 2.07% in another study,<sup>13</sup> which represents variability of 1%. In dogs, the MAC of isoflurane determined by use of the tail-clamping technique has been reported as 1.28,<sup>16,17</sup> 1.38,<sup>18</sup> and 1.39%.<sup>19</sup> Values of 1.62 to 1.68% have been reported<sup>20</sup> for dogs when electrical stimulation was used to determine the MAC. Thus, these results represent variability of up to 24%.

Variations in MAC values are usually  $< 20\%$  for most species and  $< 10\%$  within the same animal.<sup>3,21</sup> However, MAC values differ by  $> 20\%$  in some circumstances, and it is possible that such variability only applies when studies are conducted by the same investigators or performed in accordance with the same standardized method, which provides consistency in the assessment of gross purposeful movement and type of noxious stimulus applied. In addition, differences in age, temperature, and circadian rhythm could also contribute to this variation.<sup>3</sup>

Analysis of results of the study reported here indicated that clamping and electrical stimulation are both supramaximal stimuli. There was not a significant difference between techniques and site of application, although it appears that electrical stimulation resulted in higher MACs than did clamping techniques in dogs for both inhalation anesthetics (approx difference of 7% between MAC determined by electrical stimulation and clamping techniques). Conversely, MACs for isoflurane were approximately 2% higher for the clamping technique in rabbits, compared with values determined by use of electrical stimulation.

Comparison of the use of tail clamping and clamp-



ing on a vestigial digit (ie, dew claw) in pigs revealed that clamping on a dew claw, but not clamping of the tail, was a supramaximal noxious stimulus during anesthesia achieved by use of isoflurane or desflurane and that determinations with tail clamping in pigs underestimated the MAC.<sup>6</sup> Similar findings were reported<sup>22</sup> in newborn pigs between clamping on the hoof and tail during anesthesia achieved by administration of isoflurane or halothane. The opposite effect was documented<sup>2</sup> in dogs anesthetized with halothane in that tail clamping resulted in higher MAC values than did clamping on a paw. The study reported here did not reveal any significant differences between clamping the tail or paws in dogs and rabbits.

In addition, when electrical stimulation was used, we did not detect differences among the 3 sites (oral mucosa, forelimb, and hind limb) in dogs or between the 2 sites (forelimb and hind limb) in rabbits. Similar findings have also been reported for dogs in other studies<sup>4,20</sup> in which electrical stimulation was applied to the forelimbs and hind limbs.

Differences in results between our study and a study conducted by Eger et al<sup>2</sup> seem to be attributable to differences in methods used in the 2 studies. There were differences in the type of instruments used, the way in which clamps were applied to the paws, and the number of animals used. In our study, we used a sponge forcep with protective plastic tubing on the forcep jaws, and clamping of the paws involved only the third and fourth digits in 10 dogs. In the study by Eger et al,<sup>2</sup> a hemostat was used as a clamp, and it was applied across the paw or to the web between the toes in only 3 dogs. In addition, it has been documented<sup>7</sup> that even when constant pressure is applied, the actual force on the anatomic area varies as a result of changes in the nature of the tissue being compressed. We cannot compare our results with those obtained for pigs,<sup>6,22</sup> despite the fact that differences were detected among anatomic sites in all species, because there is obvious interspecies variation in reported MAC values as indicated from the results of several studies.

Skin incision was a submaximal noxious stimulus in both species. This is in agreement with results of another study in which the MAC for halothane in each of 3 dogs was 0.80, 0.52, and 0.76% (mean, 0.69%), whereas in the study reported here, the MAC for halothane was 0.75% (curve-fitting method), which is close to the value of 2 dogs in the other study. It is interesting that the MAC of isoflurane, as determined by use of a skin incision, is lower in rabbits than in dogs (0.90 vs 1.01%, respectively), despite the fact that the MAC for supramaximal stimuli is higher in rabbits than in dogs (2.04 to 2.08% vs 1.27 to 1.36%, respectively). This means that the MAC of isoflurane for the skin-incision technique is only 56 to 57% of the MAC for supramaximal stimuli in rabbits and 20 to 26% of the MAC for supramaximal stimuli in dogs. The MAC of halothane for the skin-incision technique in dogs is also only 22 to 28% of the MAC for supramaximal stimuli. Results for dogs and rabbits are in contrast with those for humans; in people, skin incision and electrical stimulation result in similar MAC values.<sup>3</sup> On the basis of these data, it is important to mention that

differences exist among species. Furthermore, these results add to the variability observed among species. Therefore, caution is advised when extrapolating results obtained from other species.

The MAC values for the various inhalation anesthetics can be decreased by concurrent use of a wide array of parenterally administered anesthetic drugs, including drugs with analgesic, hypnotic, or muscle-relaxant properties. The contribution of each of these effects is not fully understood, and drugs with analgesic properties, such as opioids and ketamine, have a sparing effect on MAC that is not necessarily more potent than the effect for nonanalgesic drugs, such as acepromazine.<sup>4,5,23-25</sup> This can be explained by the fact that gross purposeful movement is necessary for a positive response to the supramaximal stimulus; therefore, MAC determinations may involve the degree of analgesia or some degree of awareness for the response to occur.

Various types of supramaximal noxious stimulus may stimulate nociceptors to differing extents. The C-fibers respond to noxious mechanical, chemical, and thermal stimuli and are responsible for burning or aching pain,<sup>26</sup> whereas A- $\delta$  fibers respond to mechanical and thermal stimuli and are responsible for the initial pain sensation or stabbing pain.<sup>27</sup> The mechanism of action of a drug may affect 1 type of nociceptor more than another. If the supramaximal stimulus does not assess the nociceptors affected by that drug, the MAC could be overestimated. This factor is rarely considered in MAC determinations.

The investigation reported here corroborated the fact that electrical stimulation and clamping techniques are comparable supramaximal stimuli within dogs and rabbits. Skin incision is a submaximal noxious stimulus in both species, although to a differing degree for dogs and rabbits. Development of a standardized method and provision of an adequate definition for the conditions by which MAC is determined are important for obtaining reliable and repeatable results.

<sup>4</sup>S/5, Datex-Ohmeda Division, Helsinki, Finland.

<sup>5</sup>DOT-34 NRC 300/375 M1014, Datex-Ohmeda Division, Helsinki, Finland.

<sup>6</sup>Ethicon, Johnson & Johnson, Summerville, NJ.

<sup>7</sup>Sponge forcep, Robbins Instruments Inc, Chatham NJ.

<sup>8</sup>S48 Stimulator, Astro-Med Inc, West Warwick, RI.

<sup>9</sup>SigmaPlot 2001, SPSS Inc, Chicago, Ill.

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