Euthanasia of Small Animals with Nitrogen; Comparison with Intravenous Pentobarbital

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Abstract

Intravenous pentobarbital (with or without addition of saturated potassium chloride) was compared with nitrogen gas exposure for euthanasia of small animals (dogs, cats, and rabbits) in a humane society environment. Initially, electrocardiographic) and electroencephalographic monitoring were used to establish the time of death in presedated animals given either pentobarbital or exposed to nitrogen; later, nitrogen euthanasia alone was studied. Sedation with acepromazine delayed the effects of nitrogen exposure. Addition of intravenous potassium chloride dramatically enhanced the effects of pentobarbital. When sedation was omitted, nitrogen was quantitatively as effective as pentobarbital alone. An adequate flow of nitrogen is essential and the concentration of oxygen in the euthanasia chamber must be monitored.

Résumé

L'euthanasie chez les petits animaux par l'azote: comparaison avec le pentobarbital intraveineux

L'utilisation intraveineuse de pentobarbital (avec ou sans l'addition de chlorure de potassium saturé) est comparée à l'administration d'un gaz à base d'azote pour l'euthanasie des petits animaux (chiens, chats et lapins). Au début, l'électrocardiographie et l'électroencéphalographie) ont été utilisées pour établir le moment de la mort des animaux préalablement tranquillisés, ayant reçu soit du pentobarbital ou un gaz à base d'azote; par la suite, seule l'euthanasie au gaz fut étudiée. Une sédation avec l'acépromazine retarde les effets de l'administration du gaz azoté. L'injection intraveineuse de chlorure de potassium augmente beaucoup les effets du pentobarbital. Quand il n'y avait pas de sédation, l'euthanasie au gaz était quantitativement aussi efficace que celle au pentobarbital sans chlorure de potassium. Une circulation adéquate de gaz est essentielle et la concentration d'oxygène dans la chambre d'euthanasie doit être contrôlée.

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Introduction

Effective and safe euthanasia of large numbers of unwanted or sick animals is a major problem for humane societies. In 1984, the Board of Directors of the Calgary Humane Society elected to conduct a review of techniques of euthanasia used at its animal shelter with a focus on the use of nitrogen gas.

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In a veterinary practice setting, when limited numbers of pet animals are involved, intravenous administration of barbiturates or other drugs is the traditional method of euthanasia since handling of difficult or dangerous animals is not usually a problem. By contrast, the situation at the Humane Society involves a large and unpredictable number of animals, as well as variability introduced by size, breed, temperament and health status. Further, the skills and experience of the personnel available may not always be adequate to practice euthanasia by intravenous injection.

Most methods of euthanasia, using volatile or gaseous agents (1), are unsuitable in a humane society setting. Carbon monoxide is used in some centers. However, this gas, even at low concentrations, is potentially harmful to humans and should not be used in circumstances where it might escape into a closed environment; this is a particular hazard during the winter months. In addition, modified automobile engines are often used for carbon monoxide generation with potential for leaks and other hazards to the operators. For these reasons, the use of nitrogen as the euthanasic gas has appeal. Nitrogen is much safer than carbon monoxide should a leak occur, is available in tanks or as a liquid, and does not require machinery for its generation. Doubts have been expressed concerning the effectiveness of nitrogen, and in particular the immediacy of its effects, as compared with intravenous administration of barbiturates (2). Therefore, we conducted a two-part study to test the efficacy of nitrogen. Initially, intravenous sodium pentobarbital alone or in combination with intravenous potassium chloride was compared with nitrogen exposure in dogs. All animals were presedated with acepromazine. Subsequently, cats, kittens, rabbits and dogs were euthanized with nitrogen alone.

Materials and Methods

In the first part of the study, animals were divided into three groups. Group 1 consisted of four dogs, group 2 contained six dogs, and group 3 comprised nine dogs. All animals were presedated with intravenous acepromazine (Atravet — Ayerst Labs., Canada), 10 mg for those weighing less than 9 kg, and 20 mg for those over 9 kg. Hair was clipped over the chest and head to expose skin for attachment of electrocardiograph (ECG) and electroencephalograph (EEG) self-adhesive surface electrodes; the EEG electrodes were held in place by a bandage. A catheter was inserted into a cephalic vein of those animals who received sodium pentobarbital. The animals were confined in a restraining sling and the ECG and EEG were monitored continuously (Grass model 6 recorder).

Group 1 animals received an intravenous injection of 0.3 mL per kg sodium pentobarbital(Euthanol — Schering Co., Canada). Group 2 animals received a similar injection of pentobarbital followed 45 seconds later by an intravenous injection of 10 mL of a saturated potassium chloride solution. Group 3 animals were exposed to nitrogen gas in a sealed chamber.

The chamber (Snyder Mfg. Co., Denver, Colorado) measured 65 × 43 × 43 cm and was flushed with a nitrogen flow of 50 L per minute. Particular attention was paid to making certain that the chamber was leak-proof, in order that the concentration of nitrogen should rise as rapidly as possible once the gas flow was started. Monitoring of animals was continued in the chamber. Care was taken that the ECG and EEG leads did not become disconnected and leaks did not occur where the cables entered the chamber.

The concentration of oxygen in the chamber was measured continuously (Ohio 401 polarographic oxygen analyzer — Ohmeda). The analyzer was calibrated, before each group of animals was studied, with nitrogen, air and 100% oxygen. The lowest concentration of oxygen detectable by the oxygen analyzer was 4%. Commercially available nitrogen gas in tanks was used. The concentration of nitrogen in such tanks is nominally 99%, with oxygen and the rare earth gases making up the remainder. As polarographic oxygen analyzers do not respond to nitrogen or rare earth gases it was assumed that the terminal oxygen concentration in the chamber was less than 4%.

In the second part of the study, sixty-three cats, two kittens, two rabbits and five dogs were euthanized using nitrogen gas alone; no presedation was used and this precluded ECG and EEG monitoring. However, the following timed variables were recorded: reduction of the oxygen concentration in the chamber to 10%, loss of balance and collapse of the animals, respiratory arrest, dilation and fixation of the pupils, and absence of detectable heart beat. The time at which the chamber oxygen concentration fell to 10% was used as the starting time for the other variables noted above.

Additional random tests required opening the chamber as attempts were made to elicit a withdrawal reflex to toe pinching with a hemostat, palpation of the pulse, and testing for the presence of corneal or palpebral reflexes.

Results

The results of the first part of the study are shown in Table 1. In group 1 the EEG became isoelectric in an average time of 36 s (range 33–42 s). The ECG tracings became abnormal almost immediately but electrical activity persisted for an average time of 250 s (range 180–300). In group 2, the addition of potassium chloride had a dramatic effect; all ECG activity ceased within 25 s (range 17–32 s). In group 3, EEG activity persisted for an average of 959 s (range 285 s–45 min). The ECG tracings continued even longer, with an average time of 1435 s (range 660 s–51 min).

In the second part of the study, 70 of the 72 animals collapsed within 60 s (range 13–60) of the oxygen concentration falling to 10% (mean time 16 s, with a range of 11–22 s). Sixty-six of the animals had respiratory arrest within 120 s of collapsing. The two rabbits appeared more resistant to hypoxia than the other animals. Pupil dilation and fixation occurred at or near the time of respiratory arrest and preceded cessation of respiration in 12 cases. Sixty-six animals had no detectable heart beat at 360 s or less after the oxygen concentration fell to less than 10%. However, in one rabbit the heart beat persisted for 7 min, 15 s; prolonged heart beats were noted in five other animals where oxygen concentration rose when the chamber door was opened to facilitate observation. Toe and corneal reflexes were absent in 12 selected animals tested 30 s after they had collapsed. Convulsions were observed in the majority of animals following collapse; these followed a patterned sequence with opisthotonus, extension of the front legs and flexion of the hind legs, with occasional vocalization.

TABLE 1
Euthanasia of Small Animals using Nitrogen; Comparison with Intravenous Pentobarbital, with or without Potassium Chloride

	EEG (Time in Seconds to Cessation of Electrical Signal) $\bar{x}$ (range)	ECG (Time in Seconds to Cessation of Electrical Signal) $\bar{x}$ (range)
Group 1[a]	36 (33–42)	250 (180–300)
Group 2[b]	71 (47–77)	25 (17–32)
Group 3[c]	959 (285–2700)	1435 (660–3090)

[a]Pentobarbitone
[b]Pentobarbitone plus saturated potassium chloride solution
[c]Nitrogen

Discussion

The purpose of studying nitrogen gas for euthanasia was to compare its use with the currently accepted standard of intravenous injection of pentobarbital from a humane point of view. When pentobarbital is used, as in the group 1 dogs, death is not instantaneous as judged by ECG recording. This observation was highlighted in the group 2 dogs where addition of potassium chloride produced immediate and terminal results within one circulation time (i.e. 25 s). Barbiturates cause death by a combination of dose-related respiratory and cardiovascular depressant effects (1,3). As these effects are mediated through central as well as peripheral mechanisms, it is not surprising that there should be some delay as compared with potassium chloride. The latter, in saturated solution, is immediately and directly toxic to the myocardium as a result of acute electrolyte imbalance. However, potassium chloride has no anaesthetic or analgesic properties and is unacceptable for euthanasia alone (1). It may be of interest that in those states in the USA which currently have a death penalty for humans by "lethal injection", the drug combination used is: thiopental (a barbiturate), pancuronium bromide (Pavulon — a nondepolarizing muscle relaxant), and potassium chloride.

Having established a baseline for intravenous pentobarbital, it was surprising to find that the times before changes were seen in eigher ECG or EEG activity were prolonged in animals in group 3 of the first part of our study. These results did not agree with those reported by Herin *et al* (2) who found that exposure to severe hypoxia, as in our study, resulted in an isoelectric EEG in an average time of 80 s and abnormal ECG tracings after about 120 s. The only difference which appears to account for these discrepancies is that our animals were sedated with acepromazine, whereas Herin *et al* (2) used a sling restraint, prior to exposure to hypoxia. Therefore the second part of our study was undertaken to ascertain whether avoiding sedation would reduce the time to death as judged by the measured variables. Although ECG and EEG monitoring were not possible in these unsedated animals, it seems clear that avoiding sedation made nitrogen euthanasia more rapid and comparable to intravenous pentobarbital; in most animals there was no detectable heart beat at 360 s or less after exposure to nitrogen, by contrast in the pentobarbital animals ECG activity ceased within the range of 180–300 s. Although we are unable to find any reference to the protective effect of acepromazine against hypoxia, similar drugs have been used as cerebro-protective agents in humans (4).

The end point of nitrogen euthanasia as evidenced by collapse and convulsions occurred in most animals within a narrow time band. Similar observations concerning collapse and convulsions have been recorded in humans who showed these signs within 17–20 s of breathing pure nitrogen (5). A few animals exhibited tolerance to hypoxia and this was particularly so in the rabbits. Such "resistance" to hypoxia has been reported in animals below the age of four months, and nitrogen euthanasia is not recommended for this age group (1). However, it was not possible to determine the age of the rabbits and there was no relationship to age in the other "resistant" animals.

We conclude from the present study that, given certain safeguards and conditions, nitrogen euthanasia can give results similar to those seen with intravenous injection of pentobarbital. It is essential that the euthanasia chamber is leak-proof and that an adequate flow of pure nitrogen gas is used. The concentration of oxygen in the chamber must be monitored in a consistent and reliable fashion and this is best done using a polarographic oxygen analyzer. Commercially available nitrogen chambers usually are equipped with such an analyzer by the manufacturers.

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