Anesthesia for the Bottlenose Dolphin, Tursiops truncatus

Abstract. *Anesthetics can be administered to the bottlenose dolphin,* Tursiops truncatus, *by means of special ventilating equipment and intubation techniques. Nitrous oxide has been administered successfully on six occasions, and has produced definite signs of anesthesia. These developments open the possibility of performing major surgery in this species for the first time.*

The great whales, dolphins, and porpoises have recently attracted much interest, particularly because of their apparent intelligence (*1*) and large, well-developed brains (*2*). These animals belong to the order Cetacea and are air-breathing, warm-blooded mammals which spend their entire lives in an aquatic environment. Our investigations have dealt primarily with the neuroanatomy of this animal (*3*) and, especially, with the construction of a brain atlas from several thousand whole-mount brain slides. The application of classical neurophysiological techniques to the massive dolphin brain as planned by this laboratory has been hindered by two major problems: (i) no known safe anesthetics for this animal are available (*4*), and (ii) there are no adequate methods for artificially supporting respiration. These difficulties are related in part to the specialized central nervous control of the blowhole mechanisms associated with the complex engagement of the larynx into the nasopharyngeal sphincter (*5*) and with the unique pattern of respiration seen in these diving mammals. Our experiments have led to a method of anesthesia for the dolphin which has proved safe and reliable.

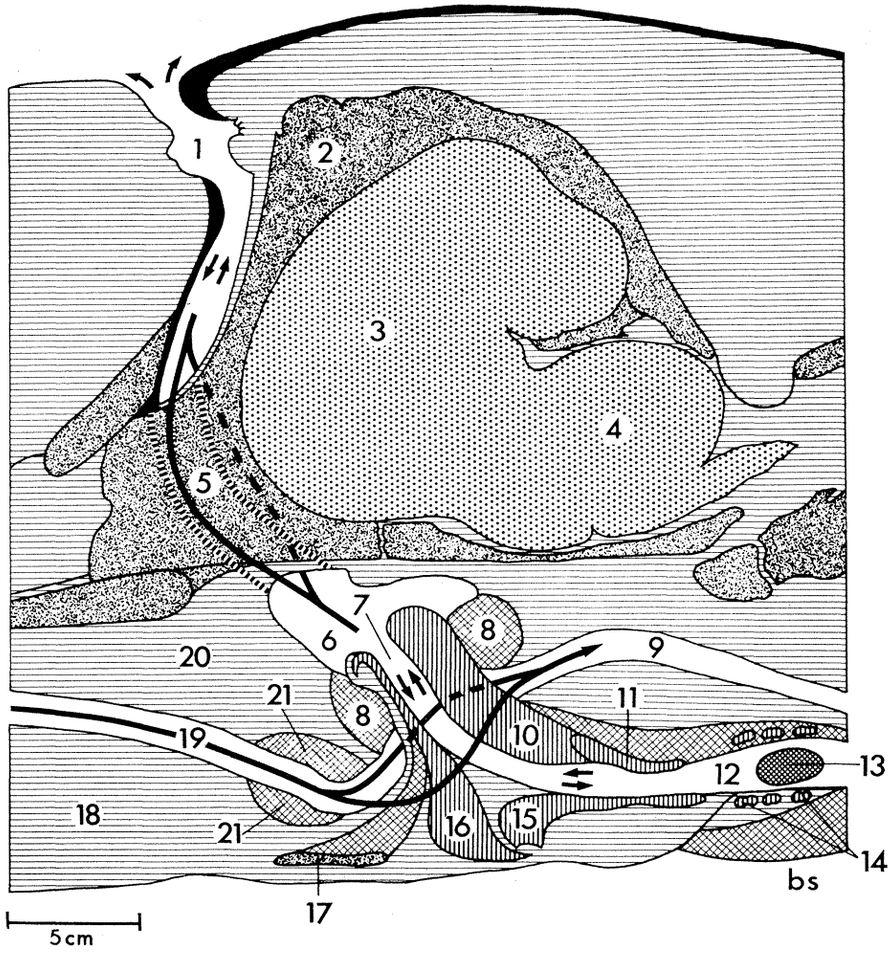

Fig. 1. Sagittal view of the head and neck region of the dolphin, *Tursiops truncatus.* Numbers indicate anatomical structures as follows: *1*, blowhole; *2*, cranium; *3*, cerebral hemisphere; *4*, cerebellum; *5*, bony nares; *6*, nasal cavity; *7*, glottis; *8*, nasopharyngeal sphincter; *9*, esophagus; *10*, arytenoid cartilage; *11*, cricoid cartilage; *12*, trachea; *13*, eparterial bronchus; *14*, tracheal cartilages; *15*, thyroid cartilage; *16*, epiglottic cartilage; *17*, hyoid bone; *18*, tongue; *19*, oral cavity; *20*, palate; *21*, oropharyngeal sphincter.

The first attempts at general anesthesia in the dolphin were made by Lilly *et al.* (*6*) in 1955. These investigators injected intraperitoneally 30 mg of pentobarbital per kilogram of body weight, the usual primate dose, and found that respiration became uncoordinated and death resulted from asphyxia. When the dose was reduced to 10 mg/kg, air leaked from the mouth, the lungs gradually deflated, and respiratory efforts became ineffectual. In a series of animals receiving various doses of pentobarbital or paraldehyde, leakage of air through the nasopharyngeal sphincter always presaged eventual respiratory failure and death.

In October 1963, we began experiments designed to develop a safe method of anesthesia for the dolphin. First we had to perfect suitable endotracheal and respiratory equipment for maintenance of adequate ventilation. Dissections were carried out to study the anatomical relationships of the oral, nasopharyngeal, and respiratory passages (Fig. 1). Manual palpation of the laryngeal-nasopharyngeal area was performed in living animals, a specially constructed surgical tank being used in which the animal was firmly secured and kept moist by hosing frequently with sea-water. Four wooden restraints were constructed to fit the contours of the body, and were placed posterior to the blowhole, anterior and posterior to the dorsal fin, and anterior to the flukes. Wet, heavy, foam padding protected the animal's body from the tank and the restraints. The use of a padded, oral speculum (Sands type for horses) allowed the dolphin complete range of jaw motion and provided adequate access to the animal's oral cavity.

For the endotracheal catheter we used a clear Tygon tube (2.5 cm outside diameter, 50 cm in length) having a short Murphy bevel and a highly distensible cuff (80 to 100 ml of air for full inflation). A heavy stainless steel coil inside the tube prevented its kinking and collapse. The final design of the respirator was based on a large-animal ventilator, the Bird mark 9X. Preparation for placement of the endotracheal tube was accomplished by insertion of the arm into the animal's oral cavity and through the oropharyngeal sphincter, so that two fingers were hooked around the vertically placed larynx. This structure was then pulled

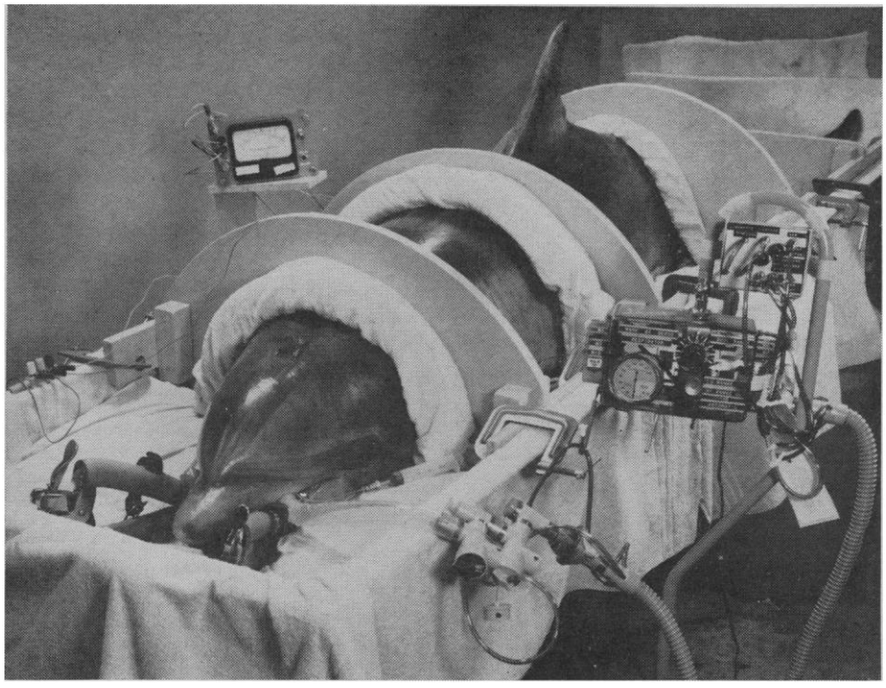

Fig. 2. An intubated dolphin connected to the respirator. The contoured body restraints, specially constructed surgical tank, oral speculum, and endotracheal tube are shown.

anteroventrally and a finger placed into the glottis for guidance of the endotracheal tube into the larynx. The animals were then maintained on air by means of the respirator (Fig. 2) for periods of up to 3 hours. Following extubation they swam, vocalized, and fed immediately on return to the home tank. As a precautionary measure, all animals were given three daily administrations intramuscularly of 1,500,000 units of benzathine penicillin G and 10 ml of vitamin B complex, with vitamin C as supportive post-anesthesia care.

The respirator was later modified by the addition of an apneustic plateau control unit which permitted variations to be made in the duration of the inflated stage. With this device, the animal was ventilated in a manner mimicking its natural pattern. Thus, the respirator rapidly inflated the animal, maintained it in an inflated state for a preset period of time, and then deflated and inflated the animal in rapid succession.

At this time the state of the animal was monitored by: (i) taking the rectal temperature by means of a telethermometer (Yellow Springs model 43) and a thermistor probe; (ii) an electrocardiogram recorded by means of a multichannel recorder (Grass model 7 polygraph); (iii) gross observations of the eye, melon, blowhole, and general body movements; (iv) observing the color of the tongue as a crude, but effective, measure of the degree of oxygen saturation of hemoglobin.

We then began experiments with various anesthetic drugs. Since we planned to map the brain in one of the studies, we hoped that barbiturate anesthesia could be utilized to enable adequate comparison with results seen in other animals. Intravenous channels were difficult to obtain in the dolphin, however, other than by means of a percutaneous puncture of the great vessels in the abdominal region. In the first experiment with a barbiturate we therefore utilized the intraperitoneal route and short-acting drugs. Thirteen milligrams of thiopental per kilogram, in two doses 5 minutes apart, followed in 10 minutes by 5 mg of methohexital per kilogram in two doses 5 minutes apart, produced a loss of all apparent reflex activity for the next 3 hours. Spontaneous movements of the eyes and flukes began 5 hours after drug administration, and voluntary respiration was first noted approximately 2 hours later. The animal was returned to the home tank 9 hours after drug administration. Leakage of air from the mouth proved to be an ominous sign, since death followed in another 1½ hours. However, the animal's lack of buoyancy in air and the increased possibility of developing hypostatic pneumonia presented an increasing risk to the animal with further maintenance on the respirator. This experiment delineated certain major problems concerned with administering anesthesia to the dolphin, namely, that any significant degree of central nervous system depression after anesthesia would probably result in the animal's death. Short of intravenous administration of drugs, further experimentation with rectal, intramuscular, intrapleural, or intraperitoneal injection of hypnotics seemed contraindicated because of uncertain absorption, metabolism, and excretion of these agents in the dolphin.

Inhalational anesthetics were tried next. Flammable agents were ruled out because of the explosion hazard in the laboratory. Potent agents were also excluded because of insufficient monitoring to insure safe, controlled administration. Halothane, therefore, was not considered because this agent depresses the cardiovascular system even in light planes of anesthesia (*7*). In seeking an agent apparently free of such side effects, nitrous oxide containing varying concentrations of oxygen was then used (*8*). Nitrous oxide is a good analgesic and amnesic agent, but a weak anesthetic (*9*). It was chosen because of the lack of side effects seen in other species of animals, including man, and because of rapid and complete recovery following its use. The depth of anesthesia is known to be dependent on the species, individual, and the premedication given. cent nitrous oxide in oxygen (*10*) was

In preliminary experiments, 50 per- administered for periods of up to 3 hours, during which time it was well tolerated. This mixture produced a definite degree of analgesia as evidenced by an elevated threshold to noxious stimuli. An uneventful recovery of the animal followed, with resumption of normal breathing pattern, swimming, and feeding activity. To secure deeper planes of anesthesia, the concentration of nitrous oxide in oxygen was then increased to 70 percent, and the following effects were noted in several experiments. Instead of passive acceptance of the respirator, the animal now attempted to breathe around or dislodge the endotracheal tube. These movements seemed uncoordinated and exaggerated during the induction phase of anesthesia. Following this brief excitation phase the animal became largely insensitive to noxious stimuli, such as subcutaneous insertions of needle electrodes or penetration of the blowhole to the nares with the fingers. Such stimuli, in our experience, have always evoked strong evasive reactions in the awake animal.

In the last three experiments we ex-

tended the monitoring procedures by utilizing the infrared CO_2 gas analyzer (Beckman) in order to measure pCO_2 in the expired air. Two penetrations of blood vessels were performed percutaneously with a 20-cm hypodermic needle. In the last such experiment, 0.25 mg of thiopental per kilogram was injected directly into the abdominal *vena cava.* This small dose of barbiturate did not appear to affect the animal adversely in any perceivable way. Monitoring devices which measure arterial pO_2, pH, and pressure will probably be necessary, however, to test safely such agents as the barbiturates or halothane when given in effective anesthetic doses. Using nitrous oxide anesthesia, we have been able to insert electrode sleeve guides into the skull on four occasions in two animals, and to record successfully cortical electroencephalographic activity. These methods open the possibility of performing major surgery in this species for the first time. However, for some surgical procedures, supplementation of nitrous oxide anesthesia with other agents may be necessary (*11*).

E. L. NAGEL, P. J. MORGANE
W. L. MCFARLAND
Communication Research Institute, 3430 Main Highway, and
Department of Anesthesiology, University of Miami School of Medicine, Miami, Florida

References and Notes

1. G. Pilleri, *Acta Anat.* **51**, 251 (1962).
2. A. S. Breathnach, *Biol. Rev.* **35**, 187 (1960).
3. M. S. Jacobs, P. J. Morgane, J. C. Lilly, B. Campbell, *Anat. Rec.* **148**, 379 (1964); M. S. Jacobs and P. J. Morgane, *Nature* **203**, 778 (1964).
4. R. S. Mackay, *Science* **144**, 864 (1964).
5. J. C. Lilly, in *Handbook of Physiology*: Sect. 4, *Environment I*, D. B. Dill, Ed. (Williams and Wilkins, Baltimore, for the American Physiological Society, Washington, D.C., 1964), pp. 741–747.
6. J. C. Lilly, *Man and Dolphin* (Doubleday, New York, 1955), pp. 35–42.
7. J. E. Mahaffey, E. A. Aldinger, J. H. Sprouse, T. D. Darby, W. B. Thrower, *Anesthesiol.* **22**, 982 (1961).
8. C. W. Fisher, L. L. Bennett, A. Allahwala, *ibid.* **12**, 19 (1951).
9. D. W. Eastwood, in *Clinical Anesthesia: Nitrous Oxide*, D. W. Eastwood, Ed. (Davis, Philadelphia, 1964), p. 25; L. E. Morris, *ibid.*, p. 74.
10. "G" cylinders containing premixed nitrous oxide in oxygen in desired concentrations were obtained from Thomas Edison Industries, West Orange, New Jersey.
11. F. Moya, *Southern Med. J.* **57**, 1091 (1964).
12. This investigation was supported in part by USPHS grant NB-03097-04, NINDB, NIH. We express thanks to Dr. John C. Lilly for his assistance and advice in these experiments, to Dr. Forrest Bird who placed a specially designed Bird mark 9X respirator at our disposal, to Benjamin J. Locke for his major contributions in many technical aspects of this work, to Drs. Frank Moya and Myron Jacobs for suggestions concerning the manuscript, to George Musil and John Richardson for the photography, and to Barbara Stolen for the drawings.

October 1964

Protein Structure Relationships Revealed by Mutational Analysis

Abstract. *Studies of tryptophan synthetase A-protein mutants have shown a structural relationship between the positions of amino acid changes associated with forward mutations and second-site reverse mutations.*

In previous mutational studies of the tryptophan synthetase system (A-gene and A-protein) of *Escherichia coli* it was shown that seven different amino acids could occupy the same position in the A-protein (*1, 2*). Two of the altered A-proteins were nonfunctional enzymatically (A-23 and A-46 A-proteins) while the others were active. One of the mutants, A-46, underwent reversion at a second site in the A-gene; this reversion event led to a change in the amino acid situated 36 residues from the position at which the original amino acid change occurred in mutant A-46 (*3*). Extensive reversion studies with mutant A-46 established that reversion only affects these two positions in the A-protein—that is, the position at which the original change occurred, and the position affected by the second-site reversion (*2*). In addition, analyses of the primary structure of six proteins from second-site revertants indicated that all the second-site changes involved the same *Tyr* → *Cys* (tyrosine, cysteine) replacement (*2, 3*). These changes at the second site must therefore reflect some functionally significant relationship between the two specific regions of the folded polypeptide chain. Thus, just as studies of "forward" mutation reveal those mutational events which lead to a loss of function, reversion analyses reveal the spectrum of mutationally permissible changes in amino acids that can restore a functional protein.

Further reversion studies were undertaken in an effort to obtain additional information on the relationship between changes in primary structure and enzyme function. It was felt that the positions at which changes in amino acids occurred as a result of reversion events might reflect structural relationships between different regions of the protein molecule. Mutant A-187 was selected for this reversion study because the position of its amino acid change was very near the position at which the change occurred in the A-46 protein (*4*) (see Fig. 1). Mutant A-187 was isolated from strain A-46 PR9, a revertant of A-46 (*4*), as shown in Fig. 1. We have now performed genetic analyses with four revertants of A-187, and have also examined their A-proteins. The results of our studies are summarized in Fig. 1. Three different reversion changes were detected. There were two revertants of the A-187 SPR2 type, in which a valine in TP3 is replaced by alanine. In A-187 SPR3 the nearby valine in TP3 is replaced by alanine. Thus substitution of alanine for either valine in TP3 of the A-protein of mutant A-187 yields a functional protein (*4*). It should be pointed out that neither of these valine residues is present in the A-protein of wild-type *E. coli.* Of great-

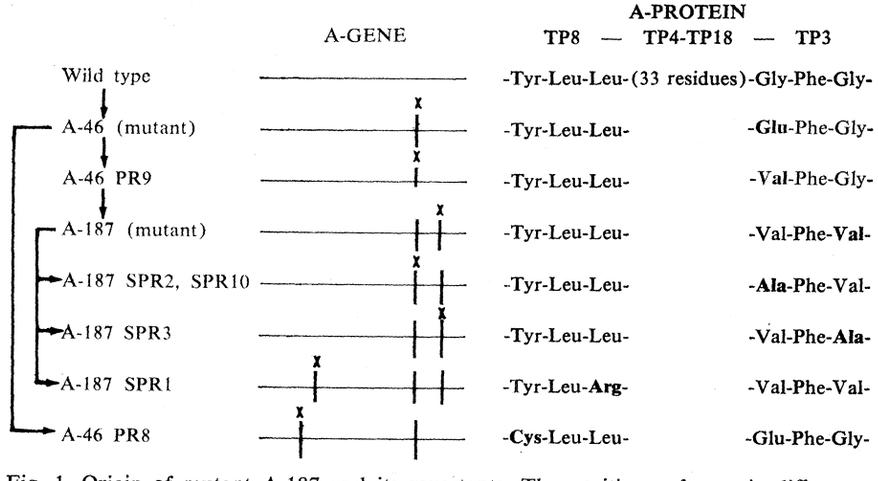

Fig. 1. Origin of mutant A-187 and its revertants. The positions of genetic differences from the wild-type A-gene and the site of each mutational change (marked X) are indicated on the left. Analyses of primary structure are given on the right; each amino acid that was changed as a result of mutation is shown in bold face type. TP8 and TP3 are two tryptic peptides of the A-protein that are joined by peptides TP4 (9 residues) and TP18 (11 residues). All the strains listed with the exception of A-46 and A-187 are prototrophic. (Abbreviations: Tyr, tyrosine; Leu, leucine; Gly, glycine; Phe, phenylalanine; Val, valine; Cys, cysteine; Ala, alanine.)