had a dose of 4000 μg only 1 day before our study. If the pertinent concentrations in liver and blood derived above are multiplied by 40, concentrations attained in vivo are similar to Cohen's values of 0.01 and 0.1 μg/ml. Even in this subject we have found no evidence of increased chromosome damage in peripheral blood leukocytes.

In this connection, it must be remembered that the dividing cells in peripheral blood cultures from normal humans are derived from mature, nondividing, circulating lymphocytes (*7*). At the time of blood collection these are almost exclusively in the G_1 or G_0 portion of the cell cycle (before DNA synthesis) (*8*, *9*). They are presumably in G_1 at the time of exposure in vivo to LSD. In Cohen's studies, the stated durations of exposure to the drug were sufficiently long to contain a portion of the DNA synthetic period, or of the stage immediately preceding mitosis (*8*). Therefore, if the effects of LSD in vivo relative to chromosome damage are restricted to stages of active synthesis or preparation for division, no chromosome damage would be expected in exposed circulating lymphocytes. Precursor cells of lymphocytes, or any other cells actively engaged in division processes, might still be affected. Without examination of other tissues of the body, this possibility cannot be ruled out.

Occasionally in squash preparations of drug users' cells, we found large cells with multiple micronuclei. Such cells were not found in control cultures. Cohen has suggested that high concentrations of LSD may be toxic to leukocyte cultures exposed to it for prolonged periods. These micronucleated cells might be the progeny of stem cells exposed to high concentrations of LSD in vivo. However, if such micronucleated cells result from previous chromosome damage, one would also expect to see some chromosome damage in cells reaching metaphase; this was not seen in our cultures.

We conclude from our work that LSD, in doses as high as 4000 μg has not been shown to damage the chromosomes of human peripheral blood lymphocytes in vivo. This conclusion is supported by observations of Petrakis (*10*). Other tissues of the body must be examined before ruling out the possibility of chromosomal damage to cells actively dividing in vivo.

Note added in proof: Since submission of this paper a comparable study supporting conclusions opposite to our own has been reported (*11*). Also, a paper has been called to our attention reporting a half time of LSD in vivo in human plasma essentially identical to that which we calculated from the mouse data (*12*).

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References

1. M. M. Cohen, M. J. Marinello, N. Back, *Science* **155**, 1417 (1967).
2. G. W. Snedecor, *Statistical Methods* (Iowa State College Press, Ames, 1956).
3. R. Schmickel, *Amer. J. Hum. Genet.* **19**, 1 (1967).
4. C. Mouriquand, J. Patet, C. Gilly, C. Wolff, *Chromosomes et Radiations: Etude in vitro de l'Action des Rayons X sur les Lymphocytes Humains, Report No. CEA-R-3007* (Centre d'Etudes Nucléaires, Grenoble, France, 1966).
5. A. Stoll, E. Rothlin, J. Rutschmann, W. R. Schalch, *Experientia* **11**, 396 (1955).
6. D. A. Kalbhen and T. W. Sargent, *Med. Exp.* **8**, 200 (1963).
7. Y. Rabinowitz, *Blood* **23**, 811 (1964).
8. M. A. Bender and D. M. Prescott, *Exp. Cell Res.* **27**, 221 (1962).
9. C. W. Gilbert and L. T. Lajtha, *Cellular Radiation Biology* (Williams and Wilkins, Baltimore, 1964).
10. N. Petrakis, in preparation.
11. S. Irwin and J. Egozcue, *Science* **157**, 313 (1967).
12. G. K. Agajanian and O. H. L. Bing, *Clin. Pharmacol. Therap.* **5**, 611 (1964).

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Anesthetization of Porpoises for Major Surgery

Abstract. *Comparison of three porpoises* (Tursiops truncatus *and* Lagenorhynchus obliquidens) *given nitrous oxide with 18 given halothane, with complete documentation of reflexes and comprehensive physiological monitoring, showed halothane to be a suitable anesthetic for major surgery while nitrous oxide was found to be inadequate. In addition, sodium thiopental administered intravenously was successfully used to facilitate intubation procedures. This development eliminated the need to intubate awake porpoises.*

The Atlantic bottlenosed porpoise (or dolphin) *Tursiops truncatus*, which has a well-developed brain (*1*) has impressed scientific investigators as being highly intelligent (*2*). Furthermore, these porpoises have a very sophisticated underwater sonar system (*3*) and highly sensitive hearing over a wide range of frequencies (*4*). The maintenance of comprehensive medical care for such valuable laboratory animals and our desire to study auditory physiology dictated the development of an anesthetic procedure suitable for major surgery.

Nagel, Morgane, and McFarland (*5*, *6*) have used 50 to 70 percent nitrous oxide (N_2O) supplemented with succinyldicholine for major surgery in the Atlantic bottlenosed porpoise *Tursiops truncatus*. Ridgway (*7*) has successfully used halothane to anesthetize the same animal.

Using the procedure recommended by Nagel, Morgane, and McFarland (*5*, *6*) we have anesthetized porpoises for surgery. Since the use of succinyldicholine and its attendant muscular relaxation preclude assessment of the depth of anesthesia by the use of reflex signs, we did not use it during our studies. Nitrous oxide was administered to three animals. To determine the degree of anesthesia, we observed the following reflexes: (i) eyelid reflex, contraction or closure of the eyelid induced by tapping on the inner canthus of the eye; (ii) corneal reflex, contraction of the eye muscles or lids when the cornea is touched; (iii) gag reflex, contraction of the throat muscles when the hand is inserted into the pharynx; (iv) tongue reflex, contraction or pulling away of the tongue when it is pulled forward; (v) anal reflex, reflex movements of any body parts when the anus is distended; (vi) swimming reflex, movements of the tail up and down in a swimming motion; (vii) pectoral scratch reflex, movements of the pectoral flippers in response to a pinprick or scratch of the chest or axillary region; (viii) blowhole reflex, movements of the blowhole when the finger is inserted into the nares of vestibular sacs; and (ix) vaginal or preputial reflex, movements of the vagina or penis or other body parts when the vagina or prepuce is distended by insertion of the fingers or other instruments.

In all three experiments with N_2O, we used a Bird Mark 9 respirator with porpoise apneustic-plateau control unit (operated as a controlled, open system), and intubation of the awake animal before administration of N_2O (*5*, *6*).

The first experiment with N_2O was performed on an adult *Lagenorhynchus obliquidens*. With intubation completed, the animal was administered 100 percent O_2 at five or six respirations per minute at a pressure of 20 mm-Hg (during respective apneustic plateaus)

for 6 minutes. Then a mixture of 60 percent N_2O and 40 percent O_2 was given for 12 minutes; no loss of reflexes occurred, and the animal's eyes would still follow a finger moved near the head. Next the N_2O was increased to 80 percent, the balance of the mixture being O_2. In 6 minutes the animal became cyanotic, and only the anal reflex could be elicited. The mixture was changed to 70 percent N_2O and 30 percent O_2, and the animal rapidly became active again. All the reflexes returned, and the animal attempted a voluntary inspiration through the blowhole. After 9 minutes on this mixture with no change in the animal's responsive condition, 60 percent ambient air and 40 percent O_2 was given for 3 minutes, the endotracheal tube was removed, and the animal resumed normal breathing. Ten minutes later the animal was replaced in the home tank, where it swam normally and ate fish.

The following procedure was then carried out on two adult *Tursiops truncatus* with the same results: Each animal was given 100 percent O_2 for 6 minutes, then 60 percent N_2O and 40 percent O_2 at a pressure of 24 mm-Hg for 28 minutes. The controlled respiration rate averaged one per 20 seconds. In this time all the reflexes remained, and the animals would still follow a finger moved near the eye. At 28 minutes, the mixture was changed to 70 percent N_2O and 30 percent O_2 for 38 minutes. No loss of reflexes resulted, and the animals continued to "follow" finger movements. Next the mixture was adjusted to 80 percent N_2O and 20 percent O_2. One hour and 10 minutes of this treatment slightly raised the threshold for the "pinprick" pectoral reflex; however, all of the animal's reflexes were present, and no cyanosis occurred. Finally, being careful to avoid causing cyanosis, we briefly administered a mixture of 90 percent N_2O and 10 percent O_2. Definite signs of anesthesia did begin to appear, but we felt it unwise to continue use of this anoxic mixture for more than a few minutes. As outlined above, successful extubation was again performed after a mixture of 60 percent ambient air and 40 percent O_2 was administered for a brief periods. Return to the home tank was uneventful.

In all three experiments, the heart beat remained normal, averaging 108 to 120 beats per minute. Apparently the cause of cyanosis in the first case was eliminated in the second two by the increase in the endotracheal tube pressure during the apneustic plateau.

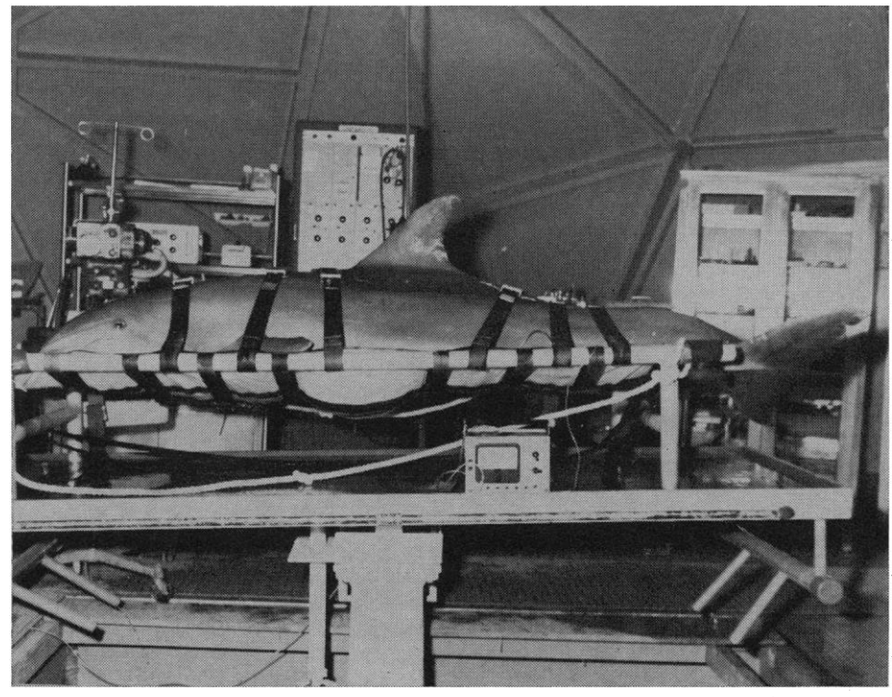

Fig. 1. Porpoise restrained with seat belts during surgery to allow freedom of tail fluke for movement in the swimming reflex. This reflex is a reliable index of the depth of anesthesia. Note outlines of tail fluke vessels which were used for blood sampling.

Our findings indicate that N_2O is inadequate for major surgery in the porpoise. Furthermore, since Nagel, Morgane, and McFarland have reported the absence of plasma cholinesterase in *Tursiops truncatus* (*6*), we feel that their preparation of N_2O supplemented with succinyldicholine is clinically contraindicated for major surgery in porpoises.

Some anesthesiologists may elect to use N_2O with a muscle relaxant other than succinyldicholine for major surgery in porpoises, their rationale being the claim that N_2O produces amnesia in humans (*8*). We do not agree with this philosophy for two reasons: (i) There is no experimental evidence for the presence or absence of amnesia in the porpoise after the administration of N_2O and (ii) we believe that to inflict pain is inhumane, whether the animal remembers it or not.

Since we were not satisfied with N_2O preparations as anesthetics for major surgery in the porpoise, the use of halothane, a more potent anesthetic, was further explored.

For the experiments with halothane more extensive physiological monitoring was available. The rectal temperature was monitored by a telethermometer thermistor probe (Yellow Spring Instruments) inserted at least 20 cm into the rectum. The P_{O_2}, P_{CO_2}, and *p*H in blood collected from central arteries and veins in the tail flukes approximately every 5 minutes were measured with an expanded-scale *p*H meter with O_2 and C_2O electrodes (*9*). Oxygen in the inspired and expired air was measured with a paramagnetic oxygen analyzer (*9*), and end tidal P_{CO_2} of each breath was measured by a Godart capnograph. The electrocardiogram was recorded on a Sanborn 350 recorder, and auditory and visual signals were presented continuously on an electrocardioscope (*9*). A urethral catheter was inserted into the urinary bladder, and the urinary output was monitored as an indirect measure of adequate kidney perfusion. The urinary output ranged from 0.7 to 1.5 ml per minute, values within the normal limits established for unanesthetized animals. The heart rates of the anesthetized animals were very regular, usually ranging from 100 to 120 beats per minute.

In the halothane experiments the Bird Mark 9 respirator and apneustic plateau control was used as a controlled open system in conjunction with the Fluotec Mark 2 vaporizer. In the first 13 cases we intubated the awake animal (*5, 6*). However, for our last five experiments we found that thiopental sodium (10 mg per kilogram of body weight) injected in the veins of the

tail flukes safely and quickly relaxed and calmed the animal, thereby greatly facilitating intubation.

The percentage of halothane to be used for induction and maintenance was determined by trial and error. On the basis of 13 successful experiments with the porpoise (three *Lagenorhynchus obliquidens*, ten *Tursiops truncatus*) we arrived at the following generalizations for the animals intubated while awake. Administration of 1.5 percent halothane resulted in a half-hour induction period before the loss of "tube bucking" and the proper depression of reflexes; administration of 2.5 percent gave a smooth induction in 15 minutes. The induction period for 3.5 percent halothane ranged from 5 to 15 minutes, and 0.75 to 1.0 percent halothane was sufficient to maintain surgical anesthesia. Occasionally an animal might require administration of 1.5 percent halothane for 2 to 5 minutes for proper maintenance during major surgery. When theopental was given before intubation, no more than 2.0 percent halothane was necessary for initial induction. Swimming movements of the free tail flukes were found to be the most reliable indication of depth of anesthesia. When these movements disappeared the subject was sufficiently anesthetized for surgery to begin. During induction, the swimming movements disappeared just after the loss of strong corneal and eyelid reflexes. The proper degree of anesthesia was maintained with the lowest concentration of halothane necessary to inhibit movement of the tail fluke. The lid and corneal reflexes were the next most dependable criteria for assessing the depth of anesthesia. All other reflexes (except anal reflex) were not prominent during periods of surgical anesthesia.

Animals were allowed to recover on 60 percent ambient air and 40 percent oxygen. In 10 to 15 minutes all reflexes except the blowhole reflex returned, spontaneous movement of the jaw and flipper occurred, and the animal's eyes followed a finger moved near them. Depending on the duration of anesthesia, the blowhole reflex returned approximately 15 to 45 minutes after the start of the recovery period. At this time extubation could be safely performed.

The swimming reflex cannot be easily observed if the porpoise is restricted with rigid wooden retainers like those used by Nagel, Morgane, and McFarland (*5*). The animal can be effectively restrained with sea belts, which do not impair the movement of the tail fluke, over the anterior and thoracic regions (Fig. 1).

Measures of gases and the *p*H of blood samples taken from the tail-fluke artery during anesthetization correlated well with corresponding adjustments of pulmonary ventilation and changes in systemic measuremens of expired CO_2. Because the central artery of the tail fluke is surrounded by a venus plexus, venous and arterial blood might occasionally intermingle during puncture of the artery or veins. However, during a recent surgical procedure for studies of cochlear potential, blood specimens were simultaneously collected from the external carotid artery and the central artery of the tail fluke, and the *p*H, P_{O_2}, and P_{CO_2} of the respective samples were found to be identical.

We have used halothane during abdominal surgery. Two ovariohysterectomies were performed on adult *Tursiops truncatus*, and an orchidectomy was performed on another adult. In each case, recovery from anesthesia was rapid and uneventful. Ten months after the surgery, the two females are completely healed, in apparent good health, and being trained for behavioral studies. The male, however, died 6 days after surgery. A postmortem examination indicated that death was caused by an infection that was aggravated by the surgery.

We have also used halothane anesthesia in the porpoise for a laprotomy, the removal of a cyst, and the repair of a corneal ulcer. Each of these animals recovered completely.

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References and Notes

1. A. S. Breathnach, *Biol. Rev.* **35**, 187 (1960).
2. S. H. Ridgway, N. J. Flanigan, J. G. McCormick, *Psychonomic Sci.* **6**, 491 (1966).
3. W. E. Evans and B. A. Powell, in *Les systèmes sonars animaux biologie et bionique*, R. G. Busnel, Ed. (Laboratoire de Physiologie Acoustique, France, Jouy-en-Josas, 1966), pp. 363–82; W. E. Schevill, and A. F. McBride, *Deep-Sea Res.* **3**, 153 (1956). W. N. Kellogg, *Porpoises and Sonar* (Univ. Chicago Press, Chicago, 1961); R. N. Turner, and K. S. Norris, *J. Exp. Anal. Behav.* **9**, 535 (1966).
4. C. S. Johnson, *Technical Paper 4178* (Naval Ordannce Test Station, China Lake, Calif. 1966).
5. E. L. Nagel, P. J. Morgane, W. L. McFarland, *Science* **146**, 1591 (1964).
6. ———, *Vet. Med. Small Animal Clinician*, **61**, 233 (1966).
7. S. H. Ridgway, *J. Amer. Vet. Med Ass.*, **147**, 1077 (1965).
8. J. H. Klock and A. Tom, *Nitrous Oxide Amnalgesia A Safe General Anesthesia for Ambulatory Patients* (Reporter Press, North Conway, N.H., 1965), p. 3.
9. Expanded-scale *p*H meter with O_2 and CO_2 electrodes, Instrumentation Laboratory Inc. (macrosample models 123 and 125A); oxygen analyzer, Beckman D2; electrocardioscope, Electrodyne PMS-5. During our most recent experiments halothane concentrations in expired air and blood were measured with a Beckman GC-2A gas chromatograph with a hydrogen flame detector.
10. We thank R. Horn and Dr. R. Ten Pas for their assistance and suggestions in the operation of the Bird Mark 9 porpoise respirator, K. Bloome and Gloria Patton for help with surgery and blood gas analysis, and Drs. W. Medway and L. Soma for reviewing the manuscript.

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Antigenic Competition: Cellular or Humoral

Abstract. *The injection of one antigen into mice inhibited the response to a second when 1 to 10 days separated the two injections. When the same type of inhibition was attempted in γ-irradiated mice reconstituted with normal spleen cells, the inhibition was greater in mice receiving 50 million spleen cells than in those receiving 10 million. The results are interpreted as favoring a humoral mechanism of inhibition.*

The injection of one antigen into an animal may partially suppress that animal's immune response to another antigen given a few days later. This phenomenon known as antigenic competition is well established. Adler (*1*) has reviewed in detail the various factors which affect its demonstration.

Antigenic competition has been explained either as a competition by the antigens for a limited number of multipotential precursor cells or as competition for a limited amount of some humoral factor. The experiments we report provide evidence that supports the humoral rather than the cellular hypothesis. However, a variation in the humoral hypothesis is advanced, namely that a humoral factor is produced during the response to the first antigen, and this factor acts as a feedback repressor of the response to the second antigen.

Adult LAF_1/J (C57L/J♀ X A/He J♂) mice from R. B. Jackson Memorial Laboratories, Bar Harbor, Maine, were used as donors and re-