

Effects of Prolonged Halothane Anesthesia on Some Cetaceans

30

*W. Medway, D.V.M., Ph.D.; J. G. McCormick, Ph.D.;
S. H. Ridgway, D.V.M.; J. F. Crump, M.D.*

SUMMARY

After prolonged halothane anesthesia (up to 24 hours) on 7 small cetaceans, variable histologic changes were found. These changes were judged to be acute, and not significant. Plasma enzyme activities monitored during the anesthesia periods also did not indicate significant liver damage.

HALOTHANE,⁸ a relatively simple organic compound,²³ is being used extensively in veterinary and human medicine as the volatile anesthetic agent of choice; and since we worked out a successful method of anesthetizing dolphins, utilizing halothane,^{11,17-19} it has become the anesthetic agent of choice not only for dolphins but for marine mammals in general.²⁰ It has many properties that make it approach the ideal in volatile anesthetic agents; however, deaths and liver changes⁹ have

been attributed to halothane in human beings.

In the course of auditory research (in the determination of cochlear potentials) involving many hours of anesthesia on porpoises, we had an ideal situation to determine whether any hepatic damage was incurred. Our opportunity was unique in that our anesthesia cases lasted as long as 24 hours. This report is an attempt to assess the degree of damage to the liver.

To date, in our opinion, evidence of pathologic changes due to halothane in lower animals has not been reported, although 2 such attempts have been made with sheep and horses.^{4,25} In a retrospective national halothane study of 856,500 human surgical procedures,²⁴ induced hepatic necrosis following single or multiple administrations was found to be a rare occurrence. Furthermore, halothane was reported to have a safety record better than an average for all anesthetic practices.

Recently, in an excellent report, a strong case was presented for the argument that halothane (in human beings and other mammals) is not a hepatotoxin, but rather is a drug which can

From the Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pa. 19104 (Medway); the Auditory Research Laboratories, Department of Psychology, Princeton University, Princeton, N.J. 08540 (McCormick and Crump); and the Naval Undersea Research and Development Center, Marine BioScience Facility, Point Mugu, Calif. 93041 (Ridgway).

This investigation was supported in part by grants to the Princeton University Auditory Laboratories from the National Institute of Neurological Diseases and Stroke, U.S. Public Health Service, and by an Auditory Laboratories contract with the Office of Naval Research. It was also aided by direct support from the Navy Marine BioScience Facility.

The authors thank Drs. D. F. Kelly of the Department of Patho-Biology, School of Veterinary Medicine, University of Pennsylvania, and J. G. Simpson of the Naval Undersea Research and Development Center, Marine BioScience Facility, for preparing the histopathology report on the porpoise liver samples; Miss L. W. Seroukis, Princeton Hospital, and Mrs. Gloria Patton, Marine BioScience Facility, for technical assistance; and Mrs. Mary Ann Gaskill of the Princeton Auditory Laboratories for transcribing surgery records and typing the manuscript.

* Fluothane, Ayerst Laboratories, Inc., New York, N.Y.

TABLE 1—Enzyme Activities, Total Serum Bilirubin, and Total Serum Protein in *Tursiops truncatus* Male (No. 1) During Halothane Anesthesia

Time (hours)*	oGT (Sigma units)	SAP (Sigma units)	Serum oGT (Sigma units)	LDH (Berger-Broida units)	Total bilirubin (mg./100 ml. serum)	Total protein (Gm./100 ml. serum)
0	200	2.4	215	1,000	0.30	8.4
1.0	1.7	230	1,330	7.9
2.0	200	2.4	210	1,130	0.30	7.6
3.0	2.3	215	1,330	7.5
4.0	2.2	230	1,420	0.60	7.3
5.0	700	1.8	305	2,000	7.3
6.5	500	1.8	555	3,350	7.3
7.0	550	2.2	920	4,850	7.5

* Time (hours) is in reference to the start of induction of anesthesia. 0 is the time at which a sample was taken from the awake dolphin.

cause a sensitization reaction.⁶ It was estimated that halothane-induced hepatitis was fatal at a rate of 1 in 10,000 cases. A well-documented case of hepatitis in an anesthetist due to halothane sensitivity has been reported.⁸ Good comprehensive reviews of the toxic and drug-induced hepatitides and of liver function in relation to surgical intervention have been published.^{7,22}

Materials and Methods

General Procedure.—Eleven dolphins were used in this study, 10 Atlantic bottlenosed dolphins (*Tursiops truncatus*) and 1 Pacific white-sided dolphin (*Lagenorhynchus obliquidens*). Of the 11, 7 were anesthetized with halothane and kept at a surgical plane of anesthesia for a number of hours (Tables 1-6) during exposure of

the inner ear and the recording of cochlear potentials. Four of the animals were used as controls, *i.e.*, they were kept out of the water unanesthetized for about 24 hours. Each of the control animals was used twice. In both experimental and control animals, blood was taken at intervals (Tables 1-10) from the vessels of the fluke or the vessels on the lateral aspect of the tail stalk. The serum was analyzed for total protein, bilirubin, and various enzyme activities; however, not all the determinations were done on all animals. On completion of the cochlear potential experiments, the animals were perfused with saline solution and Maximow's solution to obtain good tissue fixation for histologic study of the inner ear and the brain. In 4 of the halothane cases, before perfusion of the animals, liver samples were taken for histologic examination.

TABLE 2—Enzyme Activities, Total Serum Bilirubin, and Total Serum Protein in *Tursiops truncatus* Female (No. 2) During Halothane Anesthesia

Time (hours)*	oGT (Sigma units)	SAP (Sigma units)	Serum oGT (Sigma units)	LDH (Berger-Broida units)	Bilirubin (mg./100 ml. serum)	Total protein (mg./100 ml. serum)
0	0	3.2	276	2,000	8.3
2.0	0	3.2	264	1,300	Total —0.61 Direct—0.15	6.8
4.0	3.3	294	1,980	Total —0.30 Direct—0.15	7.1
5.5	0	1.9	144	940	Total —0.46 Direct—0.12	6.9
7.0	250	2.9	234	1,420	7.2
8.0	100	4.0	264	1,640	Total —0.46 Direct—0.15	6.7
9.0	400	1.9	324	1,730	7.5
13.0	350	2.8	468	3,500	7.4

All samples were hemolyzed.

* Time (hours) is in reference to the start of induction of anesthesia. 0 is the time at which a sample was taken from the awake dolphin.

TABLE 3—Enzyme Activities and Total Serum Protein in *Tursiops truncatus* Male (No. 3) During Halothane Anesthesia

Time (hours)*	OCT (Sigma units)	SAP (Sigma units)	Serum GOT (Sigma units)	LDH (Berger-Broida units)	Total protein (mg./100 ml. serum)
0	4.7	258	1,290	7.8
0.75	4.0	216	1,230	8.2
1.5	3.0	198	970	5.8
3.0	4.1	234	1,230	7.5
3.25	3.5	216	1,200	7.5
4.0	4.6	216	1,320	7.5
5.0	350	4.0	216	1,260	7.2
6.0	4.0	234	1,260	6.6
6.6	2.7	216	1,170	6.2
7.6	3.5	216	1,170	6.5
9.0	300	3.7	258	1,360	6.8
9.5	3.8	258	1,320	6.8
12.0	50	4.6	294	1,480	6.7
13.0	5.3	456	2,230	6.5
14.5	300	4.8	464	2,000	6.5
16.0	600	484	2,250	8.0
18.0	600	4.3	798	4,000	6.0

* Time (hours) is in reference to the start of induction of anesthesia. 0 is the time at which a sample was taken from the awake dolphin.

TABLE 4—Enzyme Activities and Total Serum Protein in *Tursiops truncatus* Female (No. 4) During Halothane Anesthesia

Time (hours)*	OCT (Sigma units)	SAP (Sigma units)	Serum GOT (Sigma units)	LDH (Berger-Broida units)	Total protein (mg./100 ml. serum)
0	50	7.0	660	2,450	9.6
1.0	200	7.3	600	2,000	9.2
2.0	3.0	240	1,060	5.5
2.5	6.6	480	1,400	8.1
3.0	300	7.4	540	2,000	9.3
7.0	200	7.3	540	1,700	8.1
8.0	400	5.9	600	2,000	7.1

* Time (hours) is in reference to the start of induction of anesthesia. 0 is the time at which a sample was taken from the awake dolphin.

TABLE 5—Enzyme Activities and Total Serum Protein in *Lagenorhynchus obliquidens* Female (No. 5) During Halothane Anesthesia

Time (hours)*	OCT (Sigma units)	SAP (Sigma units)	Serum GOT (Sigma units)	LDH (Berger-Broida units)	Total protein (mg./100 ml. serum)
0	200	3.7	1,300	4,400	8.4
2.5	350	2.6	1,060	3,350	7.4
3.5	250	3.0	1,140	3,650	7.6
4.75	450	3.1	1,060	4,850	7.6
7.0	200	3.3	1,170	3,200	8.1
8.0	250	3.0	1,140	3,350	7.5
10.5	700	3.2	1,140	3,500	8.0

* Time (hours) is in reference to the start of induction of anesthesia. 0 is the time at which a sample was taken from the awake dolphin.

Anesthesia.—As already mentioned, the details of our anesthesia procedure and concurrent physiologic monitoring have been reported elsewhere.²¹ Briefly summarized, induction was started with 10 to 15 mg. of sodium thiopental/kg. of body weight administered intravenously. The animals were intubated after the thiopental administration. Respiration was maintained with a

controlled open system, and induction was completed with 1.5 to 2.0% halothane. Maintenance of anesthesia was carried out with 1.0 to 1.5% halothane.

Surgery.—A preliminary report of the surgical procedure and results of the cochlear potential measurements has been made.²² Major surgical trauma consisted of: (1) ligation of both the right external

TABLE 6—Enzyme Activities, Total Serum Bilirubin, and Total Serum Protein in *Tursiops truncatus* Female (No. 6) and Male (No. 7) During Halothane Anesthesia

Time (hours) ^a	Serum GOT (Karmen units ^b)	SAP (King-Armstrong units ^b)	LDH (Wacker units ^b)	oCr (Nanomoles CO ₂ ^b)	Total bilirubin (mg./100 ml.)	Total protein (Gm./100 ml.)
FEMALE (NO. 6)						
0	155	33	130	0	0.2	9.0
3	85	37	120	0	0.1	5.9
9	120	165	0	0.3	7.4
12	160	37	205	0	0.3	7.8
21	410	36	920	23	0.4	8.2
MALE (NO. 7)						
0	195	74	262	0.5	0.2	9.3
3	190	71	275	0.2	0.3	7.9
7	130	57	200	1.3	0.3	7.8
10	180	52	295	0.9	0.4	7.8

and right internal carotid arteries (neither of which supply blood to the functional ear or brain); (2) partial removal of an arterio-venous plexus around the ear; (3) transection of the mastohumeralis muscle; (4) transection of the sternomastoideus muscle; and (5) removal of the ventral margin of the styloid process of the exoccipital bone.

TABLE 7—Enzyme Activities in *Tursiops truncatus* Female (No. 8)—Control Animal (Out of Water, No Anesthesia)

Hour	Serum GOT (Karmen units ^b)	Serum GPT (Henry units ^b)	oCr (Nanomoles CO ₂ ^b)	LDH (Wacker units ^b)
TRIAL 1				
0	133	70	4.7	274
12	200	75	4.0	252
22	230	92	7.2	293
TRIAL 2				
0	232	35	3.6	87
12	111	35	3.2	144
23	219	44	9.8	144

TABLE 8—Enzyme Activities in *Tursiops truncatus* Female (No. 9)—Control Animal (Out of Water, No Anesthesia)

Hour	Serum GOT (Karmen units ^b)	Serum GPT (Henry units ^b)	oCr (Nanomoles CO ₂ ^b)	LDH (Wacker units ^b)
TRIAL 1				
0	194	67	1.0
1	176	72	6.4
3	194	83	5.7
5	206	38	4.0
7	200	49	3.0
16	97
18	214	50	1.5
20	194	63	0.6
TRIAL 2				
0	170	48	0.1	334
12	74	2.7	334
23	106	52	0.1	272

TABLE 9—Enzyme Activities in *Tursiops truncatus* Female (No. 10)—Control Animal (Out of Water, No Anesthesia)

Hour	Serum GOT (Karmen units ^b)	Serum GPT (Henry units ^b)	oCr (Nanomoles CO ₂ ^b)	LDH (Wacker units ^b)
TRIAL 1				
0	146	37	2.7
2	162	47	4.2
4	175	53	3.9
6	178	52	2.5
8	176	54
10	180	54	1.7
12
14
16
18	166	48	3.5
20	203	50	2.7
22	182	50	2.2
24	184	50	2.1
TRIAL 2				
0	149	28	0.9	144
12	141	21	4.7	169
22	164	26	0.9	120

TABLE 10—Enzyme Activities in *Tursiops truncatus* Female (No. 11)—Control Animal (Out of Water, No Anesthesia)

Hour	Serum GOT (Karmen units ^b)	Serum GPT (Henry units ^b)	oCr (Nanomoles CO ₂ ^b)	LDH (Wacker units ^b)
TRIAL 1				
0	88	17	0.4	193
2	90	0.5	161
4	77	34	0.4	195
6	90	20	0.4	197
8	70	12	0.0	160
10	80	27	0.0	129
12	75	0.0	193
14	82	20	0.0	205
16	83	15	0.0	215
18	0.0
20	28	6.9	175
22	77	26	9.2	155
24	77	26	0.0	161
TRIAL 2				
0	95	29	0.1	201
12	250	37	0.4	215
24	120	33	0.1	235

Clinical Tests.—The chemical studies on 4 bottle-nosed dolphins (No. 1-4) and 1 white-sided dolphin (No. 5) were carried out in the Clinical Laboratory, School of Veterinary Medicine, University of Pennsylvania. The studies on the remaining dolphins were done at the Naval Undersea Research and Development Center, Marine BioScience Facility, Point Mugu, Calif.

Kits^b were used for the enzyme assays performed at the University of Pennsylvania. These tests were: serum glutamic oxalacetic transaminase (GOT) No. 505, ornithine carbonyl transferase (OCT) No. 108, serum alkaline phosphatase (SAP) No. 104, and lactic dehydrogenase (LDH) No. 500. A refractometer^c was used to determine the total protein. Bilirubin was assayed by a modification² of the procedure of Malloy and Evelyn.¹⁰ The procedures for the enzyme assays done in California are indicated in the tables. Hemogram studies were made on the blood of all animals prior to surgery. Two bottle-nosed dolphins (No. 1 and 2) had leukocytosis, and the white blood cell (WBC) counts of all others were within normal limits.¹⁴

Results

Plasma Enzyme Results.—The results of the chemical determinations on blood are given (Tables 1-10). The units of assay in some instances (Tables 6-10) differ from those of others (Tables 1-5). This is due to the fact that different laboratory facilities were available to us during operations performed at Princeton University and operations performed at the Marine BioScience Facility in California.

Histopathologic Findings.—In regard to bottle-nosed dolphins No. 2, 3, and 4 and the white-sided dolphin No. 5, the following histologic changes were noticed in liver specimens: (1) inflammatory cell infiltration (mononuclear and polymorphonuclear leukocytes) into parenchyma, often with accompanying increases in number of Kupffer's cells; (2) varying degrees of fatty vacuolization of hepatocyte cytoplasm in all dolphins except No. 3; (3) vascular congestion in dolphins 2,

4, and 5; (4) mild bilirubinosis, with some phagocytosis of pigment by reticulo-endothelial cells (there was no evidence of bile stasis) in dolphins 3 and 5; (5) hazy, indistinct vacuolization of hepatocyte cytoplasm in dolphin 3 (we are not certain of the interpretation, this probably is glycogen deposition); and (6) inflammatory cell activity and suggestions of early bile duct proliferation in portal areas of dolphin 3. There were no changes in any of the specimens indicative of chronicity. Liver lesions were most severe in dolphin 5 and progressively less severe in dolphins 2, 3, and 4. All the liver specimens in this study were compared with normal liver samples previously collected at the Marine BioScience Facility.

Discussion

Although the results in Tables 1-6 are believed to be due to the halothane, the possible effect of the anesthetic induction dose of sodium thiopental cannot be discounted.

Another factor which we cannot discount is the administration of small doses (4 to 8 mg.) of dexamethasone sodium phosphate.⁴ There was no apparent correlation of enzyme activity changes and the time of administration of the dexamethasone.

Hepatotoxins can cause microscopically evident liver injury as early as one hour following exposure, but our need to perfuse the dolphins for histologic studies of the brain and ear prevented us from observing possible postoperative clinical manifestations that are characteristically delayed for 24 to 48 hours.⁶

Mild parenchymatous changes were found in dolphins 2, 3, 4, and 5. Whether these mild changes can be attributed to the halothane cannot be said. Unfortunately, sections of liver were not taken from experimental dolphins before anesthesia for comparison with postanesthesia specimens. An interesting finding in the liver of the white-sided dolphin (No. 5) was many parasite ova adjacent to the

^b Sigma Chemical Company, St. Louis, Mo.

^c American Optical Company, Buffalo, N.Y.

⁴ Decadron, Merck Sharp and Dohme, West Point, Pa.

bile ducts. The ova had thick translucent shells with a pointed eccentric spur at the narrower end. They resembled eggs of *Schistosoma* spp.

According to results of the tests conducted, the control dolphins (Tables 7-10) did not appear to suffer any ill effects from being out of the water for 24 hours. In the experimental dolphins, prolonged halothane anesthesia did not have any marked effect, if any effect at all, on the liver. The terminal increases of serum GOT and LDH activities in dolphins 1, 2, 3, and 6 were probably due to the surgical trauma and other manipulations during the course of the experiments. The separation of LDH isoenzymes might have been of some help in identifying the source of the enzyme. There was some hemolysis of cells in all the samples taken from dolphin 2. According to earlier observations (W.M.), some dolphins seem to have very fragile red blood cells. The initial high serum GOT and LDH activities in dolphins 4 and 5 cannot be explained unless they were due to the excitement of capture and restraint before anesthesia. The activity of OCT, an enzyme primarily found in the liver (although a fair amount is present in the intestinal mucosa¹) was increased terminally in dolphin 6. The upper limit of normal by the procedure used is given as 20 units. Whether a value of 23 units is significant is not known. In all other dolphins, the activity of OCT, in our opinion, was not significant.

The range of previously published protein values for the bottle-nosed dolphin¹³ agrees well with our values. In addition, recently published normal values for serum GPT, serum GOT, LDH, and SAP² activities in the bottle-nosed dolphin corroborate our out-of-water control data and the zero time preanesthetic values for dolphins 6 and 7. With the exception of the terminal values, the normal published data add weight to the argument that the enzyme activities in our *T. truncatus* (Table 6) are within normal limits.

One might speculate from the evidence found in man that a large number

of individual halothane exposures of dolphins would be necessary to determine if halothane can sensitize a dolphin. To date, we have very little information on this problem; however, dolphin 8 had a 7-hour exposure to halothane followed by a 2-hour exposure at a later date. Dolphin 11 had 2 separate 2-hour exposures, and dolphin 10 had one 3-hour exposure. None of these dolphins suffered any ill effects post-operatively.

References

1. Cohen, L.: Serum Enzyme Determinations: Their Reliability and Value. *Med. Clin. North Am.*, 53, (1969): 115-135.
2. Ducci, H., and Watson, C. J.: The Quantitative Determination of the Serum Bilirubin with Special Reference to the Prompt-Reacting and the Chloroform Soluble Types. *J. Lab. & Clin. Med.*, 30, (1964): 293-300.
3. Henry, R. J., Chiamori, N., Golub, O. J., and Berkman, S.: Revised Spectrophotometric Methods for the Determination of Glutamic-Oxaloacetic Transaminase, Glutamic-Pyruvic Transaminase, and Lactic Acid Dehydrogenase. *Am. J. Clin. Path.*, 34, (1960): 381-398.
4. Hull, M. W., and Reilly, M. G.: Effect of Repeated Halothane Anesthesia on Sheep. *Am. J. Vet. Res.*, 29, (June, 1968): 1161-1165.
5. King, E. J., and Armstrong, A. R.: A Convenient Method for Determining Serum and Bile Phosphatase Activity. *Canad. M.A.J.*, 31, (1934): 376-381.
6. Klatskin, G.: Introduction: Mechanisms of Toxic and Drug Induced Hepatic Injury. In *Toxicity of Anesthetics*. Edited by B. R. Fink. Williams and Wilkins Company, Baltimore (1968): 159-175.
7. Klatskin, G.: Toxic and Drug-Induced Hepatitis. In *Diseases of the Liver*. 3rd ed. Edited by L. Schiff. J. B. Lippincott Company, Philadelphia (1969): 498-601.
8. Klatskin, G., and Kimberg, D. V.: Recurrent Hepatitis Attributable to Halothane in an Anesthetist. *New England J. Med.*, 280, (1969): 515-522.
9. Little, D. M.: Effects of Halothane on Hepatic Function. In *Halothane*. Edited by Nicholas M. Greene. F. A. Davis Company, Philadelphia, Pa. (1968): 106-137.
10. Malloy, H. T., and Evelyn, K. A.: The Determination of Bilirubin with the Photoelectric Colorimeter. *J. Biol. Chem.*, 119, (1937): 481-490.
11. McCormick, J. G.: Relationship of Sleep, Respiration and Anesthesia in the Porpoise: A Preliminary Report. *Proc. Nat. Acad. Sci.*, 62, (1969): 697-703.
12. McCormick, J. G., Wever, E. G., Ridgway, S. H., and Palin, J.: Function of the Porpoise Ear as Shown by Its Electrical Potentials. *J. Acoustical Soc. Am.*, 47, (1970): 67-68 (abstr.).
13. Medway, W., and Geraci, J. R.: Blood

- Chemistry of the Bottlenose Dolphin (*Tursiops truncatus*). *Am. J. Physiol.*, 209, (1965): 169-172.
14. Medway, W., and Geraci, J. R.: Hematology of the Bottlenose Dolphin. *Vet. Med./Small Anim. Clin.*, 61, (1966): 557-561 (abstr.).
15. Morgenstern, S., Flor, R., Kessler, G., and Klein, B.: Automated Determination of NAD-Coupled Enzymes: Determination of Lactic Dehydrogenase. *Ann. Biochem.*, 13, (1965): 149-161.
16. Reichard, H.: Determination of Ornithine Carbamyl Transferase in Serum: A Rapid Method. *J. Lab. & Clin. Med.*, 63, (1964): 1061-1064.
17. Ridgway, S. H.: Medical Care of Marine Mammals. *J.A.V.M.A.*, 147, (Nov. 15, 1965): 1077-1085.
18. Ridgway, S. H., and McCormick, J. G.: Anesthetization of Porpoises. In *Veterinary Anesthesia*. Edited by L. R. Soma. Williams and Wilkins Company, Baltimore (1970): In press.
19. Ridgway, S. H., and McCormick, J. G.: Anesthetization of Porpoises for Major Surgery. *Science*, 158, (1967): 510-512.
20. Ridgway, S. H., and Simpson, J. G.: Anesthesia and Restraint for the California Sea Lion, *Zalophus californianus*. *J.A.V.M.A.*, 155, (Oct. 1, 1969): 1059-1063.
21. Ridgway, S. H., Simpson, J. G., Patton, G., and Gilmarton, W.: Hematologic Findings in Certain Small Cetaceans. *J.A.V.M.A.*, 157, (Sept. 1, 1970): 566-575.
22. Schaffner, F., Kozoll, D. D., and Popper, H.: The Liver. In *Physiologic Principles of Surgery*. Edited by L. M. Zimmerman and R. Levine. W. B. Saunders Company, Philadelphia (1968): 549-576.
23. Stephen, C. R., and Little, D. M.: *Halothane*. Williams and Wilkins Company, Baltimore, 1961.
24. Subcommittee on the National Halothane Study of the Committee on Anesthesia, National Academy of Sciences-National Research Council: Summary of the National Halothane Study. *J.A.M.A.*, 197, (1966): 775-788.
25. Wolff, W. A., Lumb, W. V., and Ramsay, M. K.: Comparison of Halothane and Chloroform Anesthesia in Horses. *Am. J. Vet. Res.*, 29, (Jan., 1968): 125-132.