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STUDY OF THE EFFECTS OF OIL

ON

CETACEANS

Final Report

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1.00 A STUDY OF THE EFFECTS OF PETROLEUM ON CETACEANS

Summary and Conclusions

Cetaceans occupy surface waters to breathe, and some to feed, potentially exposing them to spilled oil by contact, inhalation or ingestion. Many are high- or top-level predators, and therefore are potential accumulators of hydrocarbon residues found in contaminated prey. Despite these concerns, there are no scientific data on the effects of oil on cetaceans. Some attention has been paid to conflicting field reports and imprecise popular news accounts. In 1969, it was reported that gray whales had died as a result of an oil well blowout in the Santa Barbara Channel. Time (February 21, 1968, p. 21) described a stranded dolphin with an oil clogged blowhole and lung hemorrhage. Critical assessment of this and other accounts of the spill did not conclusively link the marine mammal deaths with the presence of oil (Simpson and Gilmartin 1970, Brownell 1971, Brownell and Le Boeuf 1971, Le Boeuf 1971).

In Alaskan waters, two killer whales, one sick and one dead, were observed in association with an oil spill (Anon. 1971) but a precise causal relationship was not established. Duguy (1978) reported the presence of petroleum hydrocarbons in the intestine of a stranded Tursiops, without evidence to suggest that oil ingestion had been responsible for the stranding and death of the animal. These observations, coupled with two accounts of whales and dolphins swimming and feeding in oil slicks (Goodale et al. 1979, Gruber 1980), represent the extent of our information on cetaceans and oil. The reports are few perhaps because oil does not adhere

to their smooth skin so as to make exposure obvious, or alternatively, cetaceans may be able to detect oil and avoid it.

We sought to determine if bottlenose dolphins, Tursiops truncatus, can detect oil, and the limits within which they would be able to do so. Two dolphins were trained to position on a fixed underwater station at a depth of 1 m, and view a short open-ended cylinder confining various materials and oils at the surface. The dolphin pressed a paddle after detecting a substance within the cylinder. If the cylinder was empty, or the substance not apparent, the animal remained stationary for 5 seconds. By testing a blindfolded dolphin with the same substances, we examined the dolphin's ability to detect oil using echolocation.

The dolphins were tested with up to 12 different petroleum substances in as many as 31 configurations. By varying the thickness of each oil slick, or using combinations of light and dark oils, we determined the threshold of their detection ability. We reduced the visual properties of each test substance to a common measureable parameter, optical density. This gave us a basis to compare the animals' response to the various oils.

We determined that under optimum conditions of light and water clarity, bottlenose dolphins can visually detect oil with an optical density greater than 0.2 to 0.34, and with repeated testing, can reliably detect substances with an optical density of 0.05 or less. In other words, they would be able to detect even a 1 mm film of dark crude oil, which has an optical density greater than 3.0. Our tests suggest that a dolphin, using echolocation alone, can detect thick (12 mm or greater) patches of heavy oil, particularly if the substance contains air bubbles as a result of churning by wind and waves. It has yet to be determined whether dolphins can see these substances at night, or in turbid water. It will also be important to determine how the visual and echolocation thresholds change with distance from these substances.

Following the detection studies, we determined whether dolphins would avoid a controlled slick of non-toxic, colored mineral oil that we knew they could detect. Three dolphins were placed individually into a sea water pen subdivided into three areas. We established their behavior patterns, then added oil to one of the areas, and compared the response of each dolphin with its previous behavior. When first introduced to the oil, each dolphin clearly avoided the oiled area for 5, 35 and 53 minutes, respectively. Within the first two hours, each dolphin contacted the oil 2, 3 and 7 times, respectively. Thereafter, they completely avoided the oil for the remaining 6 hours of the observation period, and did not contact it at all on their second 8 hour exposure, 4 days later. Each time a dolphin contacted oil, it responded overtly by abruptly diving, and quickly returning to an oil-free area, even though the mineral oil was innocuous. We might expect that they would react similarly to a substance with noxious properties. To test whether the tactile stimulus is in itself enough to evoke a response, a study should be conducted using clear oil at night, or with a blindfolded dolphin.

In the simple setting of this study, the avoidance behavior was clear and consistent. At sea, this response might be modified by social interactions, feeding, agonistic behavior, migration or human activity.

During the course of the study, there was no opportunity to observe the reaction of free-ranging cetaceans to accidental oil spills. As an attempt to fill this gap in our data, we commissioned a study by the Hubbs-Seaworld Institute on the reaction of migrating California gray whales to naturally occurring oil seeps. Four observation sites were chosen from Pt. Conception to Coal Oil Point, California, which overlooked oil-free areas and those

with active seeps. Swimming speeds, surface and dive times, and respiratory rate of small groups of whales migrating through the study areas were compared in relation to the presence and extent of oil. Typically, the whales would swim through oil, modifying their swimming speed, but without a consistent pattern. Aerial observers occasionally noted a radical change in the whales' direction when approaching oil. This was not accompanied by any change in respiratory pattern or swimming speed, and in fact may not have been a response to oil. There were some differences in the respiratory behavior of whales when in oil-contaminated areas. In oiled waters, the whales seemed to spend less time at the surface, blowing less frequently but at a faster rate. If this reaction is interpreted as an avoidance response, it suggests that gray whales can detect oil. Whales showing no response either could not detect the amount or type of oil present, or were indifferent to it. It should be noted that the comparisons are tenuous, as it was not possible to follow specific whales into and out of oiled areas. Such are the limitations of field studies.

Cetaceans in oil would be exposed to substances which can irritate skin, and especially sensitive mucous membranes of the eyes and respiratory tract. Cetacean skin is unique, having no counterpart in other mammals. The epidermis is composed of numerous tiers of viable cells, is non-glandular and has no external keratinized layer. The cells are surprisingly rich in enzymes (Geraci and St. Aubin 1979a) and vitamin C (St. Aubin and Geraci 1980), suggesting that skin may perform important hydrodynamic (Essapian 1955) and physiological functions, beyond that of a simple barrier against the sea. Any substance which affects the skin may have far reaching consequences for these animals.

We investigated the manner and extent to which petroleum hydrocarbons would affect cetacean skin. Our study of normal histology and ultrastructure served as a basis for understanding the dynamics of growth, healing and the inflammatory response. Using small cup-like discs, we placed liquid hydrocarbons on precise areas of skin for controlled periods of time. Bottlenose dolphins were the principle subjects, and other species, including a Risso's dolphin Grampus griseus, and a sperm whale Physeter catodon, were tested opportunistically. We also tested human subjects who voluntarily exposed their arms to lead-free gasoline.

After exposing the skin to crude oils and gasoline for up to 75 minutes, we determined the reaction by observation and by using a thermographic procedure to detect increased heat radiation, as one indicator of inflammation. We found that dolphin skin exposed to gasoline and crude oil turned pale gray, and otherwise showed no evidence of damage or loss of integrity. Normal color was always restored within two hours. In marked contrast, human skin became distinctly red for up to 10 days in most subjects, and could remain discolored for up to 7 months. Using the infrared monitor, we noted that the dolphins had not reacted to the gasoline, whereas humans reacted by generating heat at the site of contact.

Our histological and ultrastructural studies showed that petroleum hydrocarbons produced mild and transient damage to cells of the epidermis, primarily in the stratum externum and stratum intermedium; the stratum germinativum and dermis were affected only by long exposures (75 min.) to lead-free gasoline. Within 3 to 7 days, the cells showed signs of recovery. When we exposed the skin of a live-stranded sperm whale to crude oil and gasoline for 17 hours, the mid- and outer layers of the skin were severely

damaged, while the basal layer and underlying dermis were still unaffected. This may be some indication of the resiliency of cetacean skin, though the circumstances under which the whale was tested were such that results must be interpreted with caution.

The skin of cetaceans is often damaged by ectoparasites (Pike 1951, Humes 1964, Perrin 1969), microorganisms (Migaki et al. 1971, Geraci et al. 1979), and predators (Ridgway and Dailey 1972), as well as aggressive social encounters. To determine how petroleum hydrocarbons might affect already damaged skin, we made a number of incisions in the epidermis, deliberately contaminating some with oil, and studied the progress of healing. We observed no gross or microscopic differences in healing of uncontaminated wounds, and those made in skin which had been previously exposed to gasoline or oil for up to 75 minutes, or wounds contaminated for up to 60 minutes with crude oil. After 15 days, all wounds had completely healed, leaving only a dark black halo. We did not quantify the cellular response to the various compounds, under different conditions of exposure. This information would provide some understanding of the defense mechanisms triggered into action by the presence of oil.

We examined biochemical processes of epidermal cells for evidence of functional damage due to oil. We measured synthesis of phospholipids, which are fundamental to cell membrane structure and stability, the concentration of α-tocopherol (vitamin E), which serves to protect lipids from oxidants, the activity of creatine kinase, an enzyme involved in basic energy transfer reactions, and the rate of oxygen consumption, as a direct index of metabolic activity. We found that exposure to oil resulted in a depression of phospholipid synthesis. Measurements of creatine kinase, oxygen consumption, and vitamin E concentrations in oil-exposed skin showed no consistent pattern of change.

The oil-related changes in phospholipid synthesis may represent a biochemical expression of the ultrastructural changes in cell integrity that we observed following exposure to gasoline. Alternatively, some of the hydrocarbon fractions may have interfered with one or more of the steps in the synthesis of phospholipids. In either case, we should view this biochemical defect as possibly jeopardizing the integrity of cell membranes, with the ultimate consequences dependent on the extent and reversibility of the damage. In all of our surface contact studies, the morphological changes were reversible, even after prolonged exposure (75 min.). However, it should be determined whether persistent biochemical changes impair the functional integrity of the skin.

A cetacean surfacing in an oil spill will inhale petroleum vapors, many of which can be harmful. There are numerous reports in the literature detailing the effects of various hydrocarbon vapors on mammals, including pinnipeds. We elected to use this information as the best approach to identifying the hazards to cetaceans. Our review included a calculation of expected vapor concentration of specific hydrocarbons in the air above an oil slick. A whale or dolphin unable to leave the immediate area of the source of a spill, or confined to a contaminated lead or bay, would undoubtedly inhale some vapors. The effect would depend on the concentrations of vapors, duration of exposure, and the susceptibility of the animal. Cetaceans that are stressed by lung and liver parasites, and adrenal disorders (Geraci and St. Aubin 1979b) might be particularly vulnerable to the effects of even low levels of hydrocarbon vapors. Animals that are away from the immediate area, or exposed to oils that have weathered in the open ocean for as little as 2-4 hours would not be expected to suffer any consequences from inhalation, regardless of their condition.

Cetaceans exposed to an oil spill might conceivably ingest oil directly, or feed on contaminated prey. There are few studies on the consequences of oil ingestion in marine mammals and none establishing critical or lethal doses. We did not undertake oil ingestion studies, in favor of reviewing the literature on oil toxicity on other animals and relating these data to our understanding of cetaceans.

Ingested petroleum can be fatal if even small quantities are regurgitated and aspirated into the lungs. Cetaceans are uniquely protected from this complication by an anatomical adaptation of the larynx which reduces or eliminates the possibility of aspirating regurgitated material. We calculated the volume of fuel oil that would be acutely toxic to a cetacean. Under normal conditions in which cetaceans such as Tursiops drink a relatively small quantity of sea water (less than 1.5 L per day), the amount of oil accidentally consumed would be well below the limit of toxicity. However, if an animal panics, as stranding odontocetes sometimes do, it may ingest large quantitites of oil-contaminated water, with consequences due as much to the water as to the oil.

Repeated ingestion of small quantities of oil might pose an insidious threat. Food organisms can accumulate certain petroleum fractions, which are transferred in turn to cetaceans. The effects of such residues on marine mammals are unknown, though there is some concern that pollutants in general can affect reproductive success (Gilmartin et al. 1976, Helle et al. 1976, vanBree 1977).

To determine the extent to which these animals have accumulated petroleum hydrocarbons, we analyzed 258 tissue samples from marine mammals stranded along the Atlantic coast of Canada and the United States, for naphthalene, one of the more persistent petroleum residues. We found

detectable levels of naphthalene in most of the tissues analyzed, and highest concentrations in blubber. Mobilization of fat stores, such as might occur during migration or lactation, would produce transiently higher blood levels of naphthalene, while simultaneously making them more available for detoxification and excretion.

One of the mechanisms for detoxifying petroleum hydrocarbons involves mixed function oxidases found in the liver. We analyzed samples from cetaceans to determine the presence and activity of one of the components of this system, cytochrome P450, and found low to moderate concentrations. If this enzyme system functions as it does in other mammals, cetaceans should be able to detoxify ingested oil. Their ability to do so would be enhanced by the typical response of inducing activity of the detoxifying enzymes, when stimulated by the substance to be detoxified. It would be possible to elucidate this mechanism in cetaceans by administering a very small amount of a labelled hydrocarbon, and monitoring metabolites in the circulation and cytochrome P450 in liver biopsy samples. This would provide an understanding of the dynamics of oil metabolism in cetaceans for which our data on naphthalene and P450 serve as a base.

Mysticetes face a peculiar threat from oil spills. The hair-like fringes of baleen can become fouled even after brief exposure to oil. Furthermore, any structural or functional alterations in the baleen filter might interfere with normal feeding. We monitored water flow through fin and gray whale baleen plates before and after contaminating them with three types of crude oil. Light to medium weight oils caused transient changes in the water flow, which returned to normal within 40 seconds. Repeated oiling of the same preparation did not produce an additive effect. Bunker C, a heavy residual oil, had a more dramatic effect, restricting water flow for

up to 15 minutes. Thereafter, though the plates were still noticeably fouled, normal flow patterns were restored. These observations alleviate the concern that crude oil would irreversibly obstruct water flow through baleen. It clearly does not do so. However, the persistence of oil on the fibers may present a different problem, by directly contaminating the food organisms, or by causing them to adhere.

We monitored the rate of disappearance of oil from the fibers, and found that light oils were undetectable after 1 hour of flushing, whereas heavier fractions persisted for 15-20 hours. These are the time frames during which we anticipate the filter mechanism might be jeopardized after a feeding foray in oil. Inability to feed during this time would not in itself be critical, but may have some impact during periods when mysticetes must feed intensively. In most other situations inadvertant ingestion of crude oil would be of no consequence and the effects on filtration would be reversible. This study points to a need for examining the adherence and contamination of plankton in contact baleen fouled with oil of various viscosities.

We considered that oil may have an effect on baleen structure and composition, thereby resulting in aberrant wear or breakage. Isolated fin and gray whale baleen plates, were soaked in gasoline, crude oil, and tar for periods far exceeding those which we might expect under natural conditions. We found no changes in the tensile properties, or in x-ray diffraction characteristics. In gray whale baleen, x-ray diffraction revealed that the α-keratin component was affected by all of the petroleum treatments. Exposure to petroleum resulted in loss of lipid and leaching of trace elements from baleen. The loss of these components did not effect the tensile properties.

We conclude our summary by setting the stage for a spill of crude oil, in a region where cetaceans are feeding and interacting. It is not unreasonable to expect that some animals may be killed - those trapped in a lead or enclosed bay, or moribund from disease. For most of the animals, however, the spill would probably not pose the kind of threat that has been popularized in the media.

We have found that Tursiops, as a representative odontocete, can detect oil, and is inclined to avoid it, especially after contacting it. Any brief encounter with oil may result in mild irritation to mucous membranes of the eyes and no consequential effect on the few morphological and biochemical features of this skin that we have thus far examined. A dolphin or whale, anywhere except in the heart of a fresh spill, could not inhale enough vapor or ingest enough oil to pose any immediate threat. There may be long term consequences that are as yet undefined. These will only become apparent by monitoring animals in a perpetually contaminated environment, and by more penetrating studies into typical target sites of petroleum damage.

We are not as certain about the behavior of mysticetes toward the oil spill, as our studies were limited to brief field observations on a single species. However, we expect that their reaction to contact with oil will be similar to that of odontocetes. In contrast with toothed whales, the threat to baleen whales would not necessarily diminish with time after the spill. Residual products, tar, and weathered oils can potentially foul plates in a way that interferes with feeding. In most cases, the major effects are reversible within a few days, before affecting the animals' nutritional status. However, prolonged impairment caused by repeated fouling might diminish blubber stores, which would be essential during migration and other

periods of fasting. Such latent effects would only be detected by correlating physical condition with petroleum residues and metabolites in animals available through commercial and aboriginal whaling operations.

References

(1) Anonymous. 1971. Kodiak Islands oil pollution. Event No. 26-70. In Smithsonian Institution Center for Short-Lived Phenomena, Annu. Rep. 1970, pp. 150-153.

(2) Brownell, R.L., Jr. 1971. Whales, dolphins and oil pollution. In Biological and Oceanographic Survey of the Santa Barbara Channel Oil Spill 1969-1970. (D. Straughan, Ed.). Vol. 1, Biology and Bacteriology, pp. 255-276. Allan Hancock Found., U.C.L.A.

(3) Brownell, R.L., and B.J. LeBoeuf. 1971. California sea lion mortality: natural or artifact? In Biological and Oceanographical Survey of the Santa Barbara Channel Oil Spill 1969-1970. (D. Straughan, Ed.). Vol. 1, Biology and Bacteriology, pp. 287-306. Allan Hancock Found., U.C.L.A.

(4) Duguy, R. 1978. Researches on the mortality factors of the cetaceans on the coast of France. Aquatic Mammals 6: 9-12.

(5) Essapian, F.S. 1955. Speed-induced skin folds in the bottlenosed porpoise, Tursiops truncatus. Breviora Mus. Comp. Zoo., Cambridge, MA.

(6) Geraci, J.R., B.D. Hicks, and D.J. St. Aubin. 1979. Dolphin pox: a skin disease of cetaceans. Can. J. Comp. Med. 43 (4): 399-404.

(7) Geraci, J.R., and D.J. St. Aubin. 1979a. Tissue sources and diagnostic value of circulating enzymes in cetaceans. J. Fish. Res. Board Can. 36: 158-163.

(8) Geraci, J.R., and D.J. St. Aubin. 1979b. Stress and disease in marine mammals. In Biology of Marine Mammals: Insights Through Strandings. NTIS Report No. PB-293 890: 223-233.

(9) Gilmartin, W.G., R.L. Delong, A.W. Smith, J.C. Sweeney, B.W. DeLappe, R.W. Risebrough, L.A. Griner, M.D. Dailey, and D.B. Peakall. 1976. Premature parturition in the California sea lion. J. Wildl. Dis. 12: 104-115.

(10) Goodale, D.R., M.A. Hyman, and H.E. Winn. 1981. Cetaean responses in association with the Regal Sword oil spill. In Cetacean and Turtle Assessment Programme, University of Rhode Island. Annual Report for 1979. U.S. Dept. of the Interior, Washington. pp. XI-1 - XI-6.

(11) Gruber, J.A. 1981. Ecology of the Atlantic Bottlenosed dolphin (Tursiops truncatus) in the Pass Cavallo area of Matagorda Bay, Texas. MS Thesis, Texas A&M University. 182 pp.

(12) Helle, E., M. Olsson, and S. Jensen. 1976. PCB levels correlated with pathological changes in the seal uteri. Ambio 5: 261-263.

(13) Humes, A.G. 1964. Harpacticus pulex, a new species of copepod from the skin of a porpoise and a manatee in Florida. Bull. Marine Sci. Gulf Caribbean 14: 517-528.

(14) LeBoeuf, B.J. 1971. Oil contamination and elephant seal mortality: a "negative" finding. In Biological and Oceanographical Survey of the Santa Barbara Channel Oil Spill 1969-1970. (D. Straughan, Ed.). Vol. 1, Biology and Bacteriology, pp. 277-285. Allan Hancock Found., U.C.L.A.

(15) Migaki, G., M.G. Valerio, B. Irvine, and F.M. Garner. 1971. Tobo's disease in an Atlantic bottlenosed dolphin. J.A.V.M.A. 159 (5): 578-582.

(16) Perrin, W.F. 1969. The barnacle, Conchoderma auritum, on a porpoise (Stenella graffmani). J. Mammal. 50: 149-151.

(17) Pike, G.C. 1951. Lamprey marks on whales. J. Fish. Res. Board Canada 8: 275-280.

(18) Ridgway, S.H., and M.D. Dailey. 1972. Cerebral and cerebellar involvement of trematode parasites in dolphins and their possible role in stranding. J. Wildl. Dis. 8 (1): 33-43.

(19) St. Aubin, D.J., and J.R. Geraci. 1980. Tissue levels of ascorbic acid in marine mammals. Comp. Biochem. Physiol. 66A: 605-609.

(20) Simpson, J.G., and W.G. Gilmartin. 1970. An investigation of elephant seal and sea lion mortality on San Miguel Island. BioScience 20: 289.

(21) Van Bree, P.J.H. 1977. On former and recent strandings of cetaceans on the coast of the Netherlands. J. Saeugetierkd. 42: 101-107.

2.00 CAN BOTTLENOSE DOLPHINS TURSIOPS TRUNCATUS DETECT OIL?

Introduction

Oil can coat the pelage of seals (Davis and Anderson 1976), otters (Baker et al. 1981) and other fur bearing mammals (Brownell and LeBoeuf 1971). Cetaceans have not been observed fouled with oil, perhaps because their smooth, hairless skin does not allow oil to adhere, or perhaps because they can detect oil, and avoid it. Dolphins have acute sensory detection capabilities both visually (Pepper et al. 1972, Herman et al. 1975) and via echolocation (Belkovich and Dubrowski 1976, Murchison 1980, Nachtigall 1980). We sought to determine whether Tursiops could detect various petroleum products using these modalities.

Two dolphins were trained to position on a fixed underwater station at a depth of 1 m, and view a short open-ended cylinder confining various materials and objects at the surface. They pressed a paddle after detecting a substance within the cylinder, or otherwise remained stationary for 5 seconds. We shaped the behavior using solid objects, and then introduced oils with different visual properties to establish the range of compounds the dolphins could detect. By testing a blindfolded dolphin with the same substances, we examined the dolphin's ability to detect oil using echolocation.

Materials and Methods

The study was conducted at the Institute for Delphinid Research on Grassy Key, Florida. We used captive-born dolphins, one male ("Nat") and one female ("Tursi"), that were five and six years old and weighed 135

and 158 kg, respectively, at the start of the study in February, 1980. The dolphins were selected because both were experienced in visual discrimination studies. They were in good health, as judged from behavior, appetite, physical condition. and blood analyses.

The dolphins were maintained in a wire fence enclosure in a lagoon along the Gulf of Mexico, with ambient water temperature and salinity ranging between 11° and 27°C, and 36 and 42 ppm, respectively. They were fed smelt, Osmarus mordax, and herring, Clupea harengus as rewards during the detection test, and at the end of each day, to provide the balance of their prescribed daily ration of 5.8-7.9 kg/day. Feeding rates were calculated on the basis of approximately 60-70 Kcal/kg/day.

Apparatus

The study area consisted of a rectangular pen with one wall of solid earth fill and three of wire fencing. It was 49 m long, 12.8 m wide, with an average water depth of 4-5 m, depending on the tide. Two small, square platforms placed 5.7 m apart were hinged to a dock which ran along one side of the pen. The platforms floated on styrofoam blocks so that they maintained a constant level in water. One platform, the resting dock, was occupied by a trainer who fed the animals and controlled their behavior during a test. The second platform was the trial dock, where an examiner prepared and deployed cylinders containing the samples to be viewed by a dolphin. The examiner was isolated from the trainer and the dolphin by a roof, two solid side panels, and a front panel with 1-way windows allowing a view to the outside. Cylinders were deployed and retrieved through a small door that opened into a 3.1 x 0.6 m canal in the floor along the front of the trial dock (Figure 2.1).

We constructed 20 hollow cylinders. Each was made of 3 mm thick

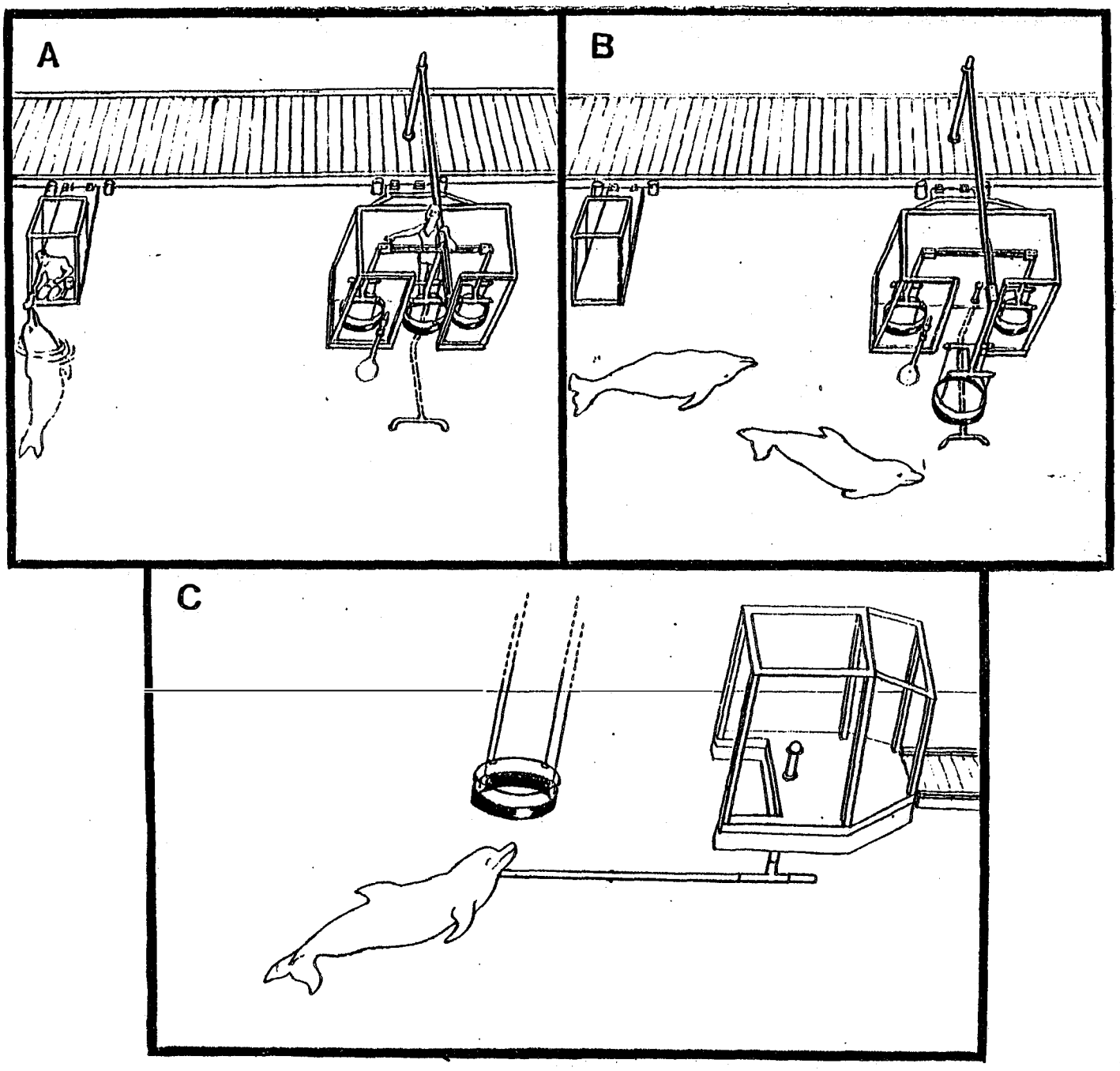

Figure 2.1

plexiglass measuring 46 cm in diameter, and 22 cm high, with an internal cross-sectional area of 1662 cm^2. A 10 cm wide black band was painted around the middle of both the inner and outer surfaces of each cylinder, to eliminate images that might be created by the oil-plastic interface. A cylinder was held half submerged by four thin wires attached to the rim, and hung from a frame that rolled along a track. Three cylinders, independently suspended in the canal, could be filled with test substances, and deployed in any order through the front door to a distance of 40 cm in front of the dock. At the conclusion of a series of trials, the test compounds were retrieved in a way that would not contaminate the water. The ends of each cylinder were capped with plywood discs fitted with gaskets made of Geocel[1] and the assembly with its contents was sandwiched tightly together with a bolt running through the middle of the discs (Figure 2.2).

To view the contents of the cylinder, the dolphin rested on a crescent-shaped plastic station 1 m below the surface so that its eyes were directly beneath the test substance. On detecting the substance, the dolphin pressed a round wooden disc triggering a spring-actuated flag inside the examination dock. Signals used to direct the dolphins through each step of the testing sequence were generated by a modified Bambino Classic Tones, Model EV-04[2] miniature electronic organ. The keyboard was rewired to provide two tones of 200 and 400 Hz and a chirping sound produced by an electronic metronome. The unit was connected to a

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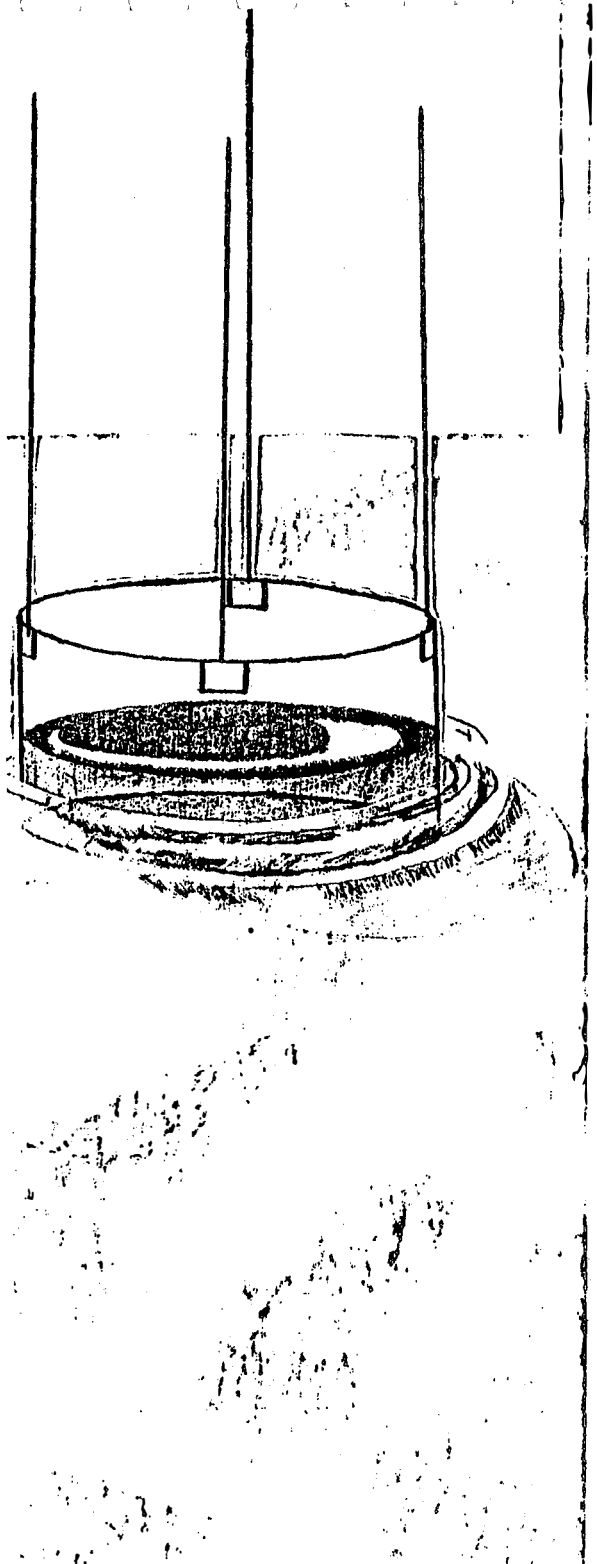
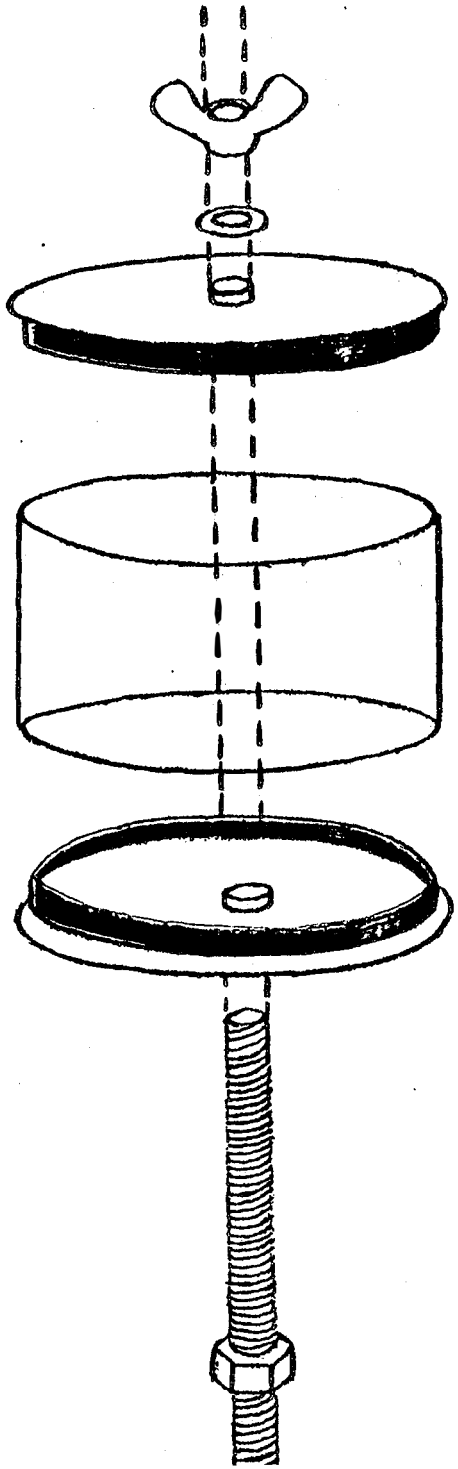
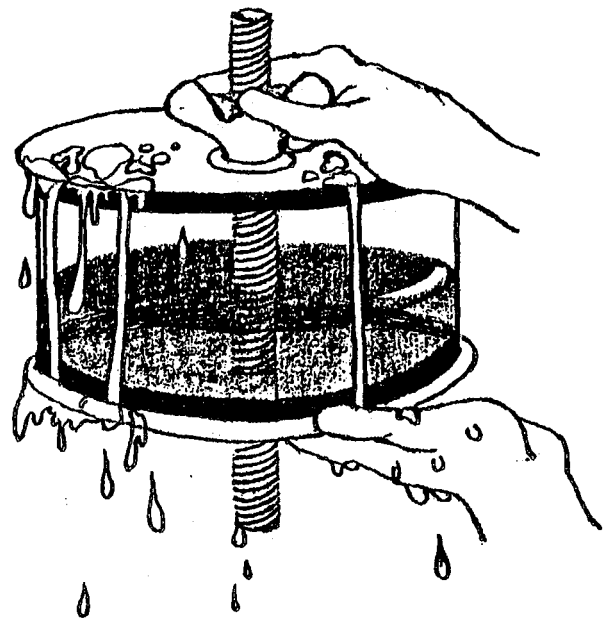
[1] Geocel Corporation, Elkhart, IN
[2] Bambino Inc., Los Angeles, CA

Figure 2.2







Realistic MPA-20[3] amplifier, driving one in-air and one underwater speaker. The latter speaker was covered on one side with closed-cell neoprene, and directed away from the sample cylinder, to minimize reflected sounds that might have given the animals supplementary information about the contents of the cylinder.

Toward the end of the visual discrimination experiment, we initiated a series of echolocation trials using the male dolphin. We fitted the animal with eyecups to prevent if from seeing the test sample, thereby forcing the dolphin to use echolocation. To familiarize the dolphin with the latex[4] eyecups, they were first placed on various locations on the dolphin's back, sides and head, gradually approaching it's eyes. The eyecups were worn for up to 30 minutes at a time, and were removed if the dolphin showed signs of discomfort. To avoid visual cues, the eyecups were removed only after the test cylinders were retrieved into the canal.

Test Substances

During training, various solid plastic and wooden objects were placed within the cylinders to establish the "go" response when objects were detected. We then substituted a disc of styrofoam that completely filled the cylinder. Fading from the detection of styrofoam to the detection of test oils was accomplished by progressively removing larger areas from the center of the styrofoam while filling the vacated area with whipped oil. Transfer occurred rapidly and after three days the cylinder was filled with test oils alone.

The oils used in the study are listed in Table 2.1. We reduced the visual properties of 34 different oils and oil mixtures to a common measurable parameter, optical density. We did this by passing light from a 100 W tungsten bulb mounted in a photo-flood reflector (Smith-Victor BD12)

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[3] Radio Shack Inc., Ft. Worth, TX

Table 2.1: Identification and source of samples used in the oil detection study.

Crude Oils	Source
Inter-Provincial	Shell Refinery, Oakville, Ont.
West Texas	Shell Refinery, Oakville, Ont.
Unidentified	Anonymous
Residual Oils	
Bunker C	Belcher Oil Co., Port Everglades, FL
beach tar	Florida Keys
Refined Motor Oils	
Quaker SAE30	Quaker State Oil Refining Co., Oil City, PA
Kendall SAE20	Kendall Refining Co., (Division of Witco Chemical Co.), Bradford, PA
Evinrude 2-cycle (50/1)	Evinrude Corp., Beloit, WI
Mineral Oil	
Adams Heavy White Mineral Oil (Stock #118)	Adams Laboratories Div., Miami, FL
Refined Fuels	
Diesel #2 (Lots A and B)	American Petrofina Marketing Inc., Port Arthur, TX
Regular gasoline (leaded)	Texas Oil Corp.

covered with a diffusing screen (Smith-Victor D120), was passed through a measured thickness of oil in a 9 cm diameter Pyrex petri dish. The optical density was measured using a photo cell (Archer 276-116 cadmium sulphide photo cell)[5] coupled to an ohm-meter 427-A[6]. The meter was calibrated on each occasion using celluloid filters of known graded optical density (#1523422 Kodak Photographic Step Tablet No. 3)[7]. The optical density of each sample was calculated by interpolating between the values of the standards. Using this method we determined the optical density of each type and thickness of oil sample used in the discrimination trials (Table 2.2, 2.3, 2.4). The coefficient of variation (SD ÷ mean), on 12 individually prepared oil samples measured up to 10 times each, was less than 1%.

Preparation for Testing

Prior to each session, an examiner would receive instructions on the nature of the test substance(s) to be used, a list of the randomly assigned numbered cylinders which were to contain the test substance (S+) or remain empty (S-), and the sequence in which they were to be presented. The sequence was generated by an Apple computer[8] randomization function, modified by the program into a Gellerman series (Gellerman 1933) such that there were never more than 3 consecutive presentations of either S+ or S- samples. Generally, only one test substance was used in a session, attempting as much as practical, to equalize the number of S+ and S- presentations. Of the 3,906 presentations, 52% were S+, and 48% were S-.

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5 Tandy Corp., Ft. Worth, TX
6 Hewlett-Packard Inc., Palo Alto, CA
7 Kodak Corp., Rochester, NY
8 Apple Computer Co. Inc., Cupertino, CA

We arbitrarily determined that an animal could detect a given substance when overall performance on both S+ and S- trials equalled 90% or better. Novel substances were introduced outright, or by interspersing them as "probe-trials", with known detectable (90% performance) substances. Probe substances were never introduced as the first or last of a series. No more than 2 probe trials were included in any series of 10 trials with a known substance. If the animal detected the probe substance, we made this the principal test substance in the next session.

Test Procedures

While the trainer interacted with the dolphin positioned in front of the resting dock, the examiner prepared one cylinder with sea water (S-) as a control, and 1 or 2 cylinders with test substances. This procedure was carried out within the confines of the canal of the trial dock. One of these cylinders was then moved into place through the door of the dock. The sound of the door closing was used as the cue for the trainer to broadcast a 200 Hz tone signalling the dolphin to swim underwater to the viewing station at the examination dock. While approaching the station, the dolphin usually examined the sample with both eyes for approximately 1 second before resting its chin on the stirrup, with head rotated and one eye fixed on the cylinder.

Once the dolphin was on station, the examiner presented a 400 Hz tone. If the cylinder contained a substance (S+), the animal was required to leave the station within 5 seconds to press the paddle. If the cylinder contained only sea water (S-), the dolphin was required to remain on station for a full 5 seconds.

For a correct response, the examiner blew a police whistle transmitted in-air and underwater, signalling the animal to return to the resting dock to receive a reward of 2 fish. If the response was incorrect, the examiner broadcast a "chirping" sound recalling the dolphin to the resting dock

without reward. This signal was also used to recall the dolphin during any breakdown in the testing sequence.

Results

Both dolphins successfully acquired the desired behavior with less than two months of training, likely because they were captive-born, had interacted with trainers within months of birth, and had been trained for four months in a preliminary phase of this study. The male, who acquired the behavior sooner and performed more reliably had also been involved for two years in a discrimination study. The female had completed most of the visual study when she was removed from the program in June, 1981, because of an eye injury unrelated to the experiment.

A total of 3,906 visual and acoustic trials were conducted, of which 2025 were test trials. These were done generally in blocks of 10 trials per session, once or twice daily. Four examiners were involved in the major portion of the study; with no significant difference in performance associated with the examiner ($X^2 = 4.19$; $P < 0.05$). A fifth examiner entered the program toward the end of the study, when the dolphins were being tested at threshold levels. The dolphins' response was poorer with this examiner, likely because the test substances were more difficult to detect. The dolphins performed the visual trials on all S+ and S- samples with an overall accuracy of 95%. The male performed the acoustic trials with 79% accuracy. We established peak performance as the maximum that could be attained using known detectable substances. For visual trials it was 100% for both dolphins, and for acoustic trials 91% for the male. We arbitrarily and conservatively accepted 90% or better of peak performance as a clear indication that the animals could reliably detect a substance, and below 75% as an indication that they could not detect it at all.

The performance on the visual detection studies for both dolphins are shown on Tables 2.2-2.4. The female detected 6 mm thick slicks of crude oil and three refined motor oils, but could only reliably detect diesel oil thicker than 25 to 31 mm (OD > 0.12). To quantify the limits of her visual detection, we tested 6 mm slicks of graded mixtures of blue-tinted Evinrude 2 cycle motor oil that she could detect, and amber-colored diesel fuel that she could not detect. Her threshold for the mixtures (OD = 0.20) was higher than for diesel alone (OD = 0.12 to 0.16). In other words, she detected the blue-tinted mixed oil less reliably than she could a thicker slick of amber-colored diesel with the same optical density.

The male detected 6 mm slicks of 3 crude oils, 2 residual oils, and 3 refined motor oils (Table 2.3). All had an optical density greater than 0.26. Initially, he did not detect the same thickness of gasoline or diesel fuel (OD $\leq$ 0.05). He could however, detect a diesel slick of 17 mm or thicker (OD $\geq$ 0.08). As with the female, the male detected the mixed oil less reliably than the diesel with the same optical density.

When we continued to test the male at threshold levels, we noticed that he eventually began to detect lighter substances (Table 2.4). For example, after a 3 month interruption in the visual threshold studies, he was retested with diesel fuel, gasoline, mineral oil, and various mixtures. He reliably detected diesel slicks as thin as 3 mm (OD < 0.05), and oil mixtures down to an OD of 0.07. Previously unable to detect 6 mm thick samples of gasoline (OD < 0.05), he now detected them 80% of the time. Mineral oil, virtually transparent, was still not detected.

While analyzing the results of this re-testing, we noted a distinct difference in performance between morning and afternoon trials when the male was presented with the Evinrude: mineral oil mixture (OD = 0.12).

Table 2.2: Performance of the dolphin "Tursi" in trials to determine visual detection of various oils and fuels.

Sample[a]	OD[b]	Trials		Probe Trials[c]	
		Number	Percent Correct	Number	Percent Correct
Crude Oil					
unidentified	> 3.0	30	97		
Refined Motor Oils					
Quaker SAE 30	0.26	32	100		
Kendall SAE 20	0.26	30	93		
Evinrude 2-cycle	0.79	54	93		
Refined Fuel					
Diesel A 35 mm	0.20	15	100		
31 mm	0.16	28	96		
25 mm	0.12	16	63		
Mixed Oils					
Evinrude: Diesel A					
47.1 : 52.9	0.64	32	100		
24.9 : 75.1	0.50	30	100	2	100
15.4 : 84.6	0.34	44	95	8	100
5.6 : 94.4	0.20	38	80	4	0

[a] All samples were 6 mm thick, unless otherwise noted.
[b] Optical density, determined using a photocell apparatus (refer to text).
[c] A probe trial is one in which a sample which is not the principle test substance is interspersed into a testing sequence (refer to text).

Table 2.3: Performance of the dolphin "Nat" in trials to determine visual detection of various oils and fuels.

Sample[a]	OD[b]	Trials		Probe Trials[c]	
		Number	Percent Correct	Number	Percent Correct
Crude Oil					
Inter-Provincial	> 3.0	63	98		
West Texas	> 3.0	38	100		
Unidentified	> 3.0	29	93		
Residual Oil					
Bunker C	> 3.0	30	100		
Beach tar	> 3.0	30	97		
Refined Motor Oils					
Quaker SAE 30	0.26	151	98	4	100
Kendall SAE 20	0.26	16	100		
Evinrude 2-cycle	0.79	75	100	2	100
Refined Fuel					
Diesel A 35 mm	0.20	30	100		
25 mm	0.12	17	100	4	75
17 mm	0.08	23	100		
6 mm	0.05	—	—	11	27
Leaded gasoline	0.05	—	—	4	0
Mixed Oils					
Evinrude : Diesel A					
47.1 : 52.9	0.64	30	100		
24.9 : 75.1	0.50	30	100	2	100
15.4 : 84.6	0.34	30	100	2	100
5.6 : 94.4	0.20	49	100	3	100
2.6 : 97.4	0.12	28	89	4	100
1.0 : 99.0	0.08	18	83		

[a] All samples were 6 mm thick, unless otherwise noted.
[b] Optical density, determined using a photocell apparatus (refer to text).
[c] A probe trial is one in which a sample which is not the principle test substance is interspersed into a testing sequence (refer to text).

Table 2.4: Performance of the dolphin "Nat" in trials to determine visual detection, after repeated testing at threshold.

Sample[a]	OD[b]	Trials		Probe Trials[c]	
		Number	Percent Correct	Number	Percent Correct
Diesel B 12 mm	0.09	14	100		
6 mm	0.06	128	94	4	4
4.5 mm	< 0.05	30	100		
3.0 mm	< 0.05	14	100	3	60
1.5 mm	< 0.05	33	76		
0.6 mm	< 0.05	—	—	4	0
Leaded gasoline	< 0.05	40	80	7	43
Evinrude: Mineral Oil (6 mm)					
9.7 : 90.3	0.20	26	96		
6.4 : 93.6 (a.m.)	0.12	76	93		
6.4 : 93.6 (p.m.)	0.12	17	65		
4.7 : 95.3	0.09	26	92	4	25
4.0 : 96.0	0.07	25	88	4	25
3.4 : 96.6	0.06	—	—	3	0
Evinrude: Diesel B (6 mm)					
1.0 : 99.0	0.08	42	98		
Mineral oil (12 mm)	0.05	7	29	10	10

[a] All samples were 6 mm thick, unless otherwise noted.
[b] Optical density, determined using a photocell apparatus (refer to text).
[c] A probe trial is one in which a sample which is not the principle test substance is interspersed into a testing sequence (refer to text).

This could profoundly influence the dolphins ability to detect samples at threshold limits. For example, the male could reliably detect this mixture (93% correct), but not in the afternoon (65% correct). Since the study had terminated at the time of this finding, we did not attempt to determine whether the animal's performance was affected by ambient light, clarity of the water, or a peculiarity in the animal's behavior. There were no comparable differences noted when testing any other substances. We determined the male's ability to detect oils by echolocation, by shaping the behavior using the styrofoam disc in the cylinder. In 97 trials over a period of 2 months, his response to both S+ and S- was 91% correct. Thereafter when presented with test substances, he detected 12 mm thick films of Inter-Provincial and the unidentified crude oil, as well as Bunker C and mineral oil, though the latter two were more reliably detected when they churned and contained air bubbles. Other oil samples of that thickness were not reliably detected. He was unable to detect any sample of 6 mm thickness (Table 2.5).

Toward the end of the study, the male was tested in a series of 77 trials in which he was required to detect 12 mm thick samples either visually or acoustically according to a randomized scheme. His performance in this series showed that he detected diesel fuel visually, but not by echolocation, whereas the converse was true in the mineral oil trials (Table 2.6).

Table 2.5: Performance of the dolphin "Nat" in trials to determine acoustic detection of various oils.

Sample	Trials		Probe Trials[a]	
	Number	Percent Correct	Number	Percent Correct
Styrofoam	97	91		
Bubbled Oils - 12 mm				
Bunker C	30	88		
Mineral	18	94		
Crude and Refined Oils - 12 mm				
Inter-Provincial	81	86	1	100
Unidentified	58	84	2	0
West Texas	16	56		
Bunker C	133	73	8	50
Diesel	22	64		
Mineral	59	71	5	60
Crude and Refined Oils - 6 mm				
Inter-Provincial	4	25		
Unidentified	13	62		
Diesel	6	33		

[a] A probe trial is one in which a sample which is not the principle test substance is interspersed into a testing sequence (refer to text).

Table 2.6: Detection performance of a male bottlenose dolphin (Nat) in paired visual and acoustic trials using 12 mm slicks.

	Visual		Acoustic	
Sample	Total	% Correct	Total	% Correct
Diesel	14	100	16	71
Mineral Oil	7	29	15	87
Bubbled Mineral oil	7	43	18	94

Discussion

Bottlenose dolphins in this study visually detected oil. The darker the substance, the easier it was to detect. We conservatively determined the critical threshold to be D = 0.2 to 0.34, which is much lighter than the optical density of even thin films of dark crude oil[9].

Thus, these dolphins would be able to see a spill of crude oil at its source, and most weathered oils that darken and form dense aggregations. Crude oil dispersed into a thin sheen (< 0.1 mm) would not be easily detected, nor would any refined products such as gasoline, diesel fuel, and solvents that tend to disperse into thin films very rapidly. Yet dolphins might eventually learn to detect some of these substances after exposure in a contaminated environment. The rate of learning would probably be enhanced if the dolphin experienced pain or discomfort on contact, as seems to have been apparent in our oil avoidance study (see Section 3.0, this report).

Under circumstances when vision is impaired, an animal may be able to rely on echolocation for detecting thicker high viscosity substances, such as residual or weathered oils, or any oil churned by wind or wave action so as to entrap air bubbles. Yet in our study, the dolphin's ability to detect oil using echolocation was not as effective as when using vision. This may have been a fault of the experimental design. The dolphin was only trained over a brief period and perhaps an insufficient number of trials. The positioning of the animal in front of the target had been established to optimize visual contact with the oil, and may not have been the most advantageous position for echolocation. Furthermore, the test

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[9] We found the optical density of 1 mm thicknesses of three types of crude oil to be greater than 3.0

substances were confined in a cylinder, so that the animal did not have the opportunity to examine the edge of the oil slick; where the contrast between oil and water may have given him an important cue.

Additional studies would have been required to determine more precisely the features of the environment, and of a dolphins' sensory system that influences it's ability to detect oil. We did not determine the distance at which a dolphin could no longer detect oil, nor did we test the dolphins under conditions of poor water clarity. Color may influence an animal's ability to detect oil, particularly at threshold levels. Our data showed that on first exposure, blue-tinted Evinrude mixtures were less detectable than amber-colored diesel fuel of the same optical density. The difference in detectability was far less pronounced after learning. Though dolphins have little or no color vision, they appear to have greater sensitivity to light in the blue-green portion of the spectrum (Madsen and Herman 1980). Therefore, we expected them to see the Evinrude mixtures more readily. Their ability to do so may have been impaired by the lack of contrast when viewing the blue sample against the sky. As a natural extension of our study, the effects of these variables should be determined, along with the dolphin's ability to detect oil slicks at night.

References

(1) Belkovich, V.M. and N.A. Dubrowski. 1976. In Sensory Bases of Cetacean Orientation. Nauka, Leningrad.

(2) Gellerman, L.W. 1933. Chance orders of alternating stimuli in visual discrimination experiments. J. of Genetic Psych. 42: 207-208.

(3) Herman, L.M., M.F. Peacock, M.P. Yunker and C.J. Madsen. 1975. Bottlenosed dolphin: double-slit pupil yields equivalent aerial and underwater diurnal acuity. Science, 189: 650-652.

(4) Madsen, C.J. and L.M. Herman. 1980. Social and ecological correlates of cetacean vision and visual appearance, pp. 101-148. In Cetacean Behavior: Mechanisms and Functions (L.M. Herman, Ed.). J. Wiley, New York, NY.

(5) Murshison, A.E. 1980. Detection range and range resolution of echo-locating bottlenose porpoise (Tursiops truncatus). In Animal Sonar Systems (R.G. Busnel and J.F. Fish, Ed.). Plennum Press, New York, NY.

(6) Nachtigall, P.E. 1980. Odontocete echolocation performance on object size, shape and material. In Animal Sonar Systems (R.G. Busnel and J.F. Fish, Ed.). Plennum Press, New York, NY.

(7) Pepper, R.L., F.A. Beach, J.V. Simmons and P.E. Nachtigall. 1972. In-air visual acuity of the bottlenose dolphin. Proceedings of the Ninth Annual Conference on Biological Sonar and Diving Mammals. pp. 83-89. Stanford Research Institute Press.

3.00 OIL AVOIDANCE BY CAPTIVE BOTTLENOSE DOLPHINS

Introduction

The ultimate consequences of an oil spill on a cetacean will depend in part on the degree and duration of exposure. It is therefore important to know whether they will avoid oil that they can detect. Two accounts of cetaceans in the midst of oil slicks (Goodale et al. 1979, Gruber 1980) suggested that the animals did not avoid the oil. The observers were unable to determine whether this was the natural response, or if their instinct to avoid the slick was over-ridden by feeding behavior which was occurring at the time of the observations.

In this study, we sought to determine whether three captive bottlenose dolphins, under controlled conditions, would avoid a detectable slick of colored, non-toxic mineral oil. We did this by confining them to a pen subdivided into three areas. After establishing their patterns of behavior, we added oil to one of the areas, and compared the response of each dolphin with its previous behavior.

Materials and Methods

Subjects

Three female dolphins aged approximately 4 years were captured in the Gulf of Mexico near Biloxi, MS in July 1980 and transported to the Institute for Delphinid Research, Grassy Key, FL. They were maintained together in sea water pens, and fed a daily ration of herring (60-70 Kcal/kg body weight). The dolphins were in good health as judged by routine clinical examination, feeding behavior, and blood studies.

These dolphins were not trained to perform any special tasks, but they were used for skin-contact studies for 9 months prior to this experiment

(see Section 6.0, this report).

Test Area

The test pool was a rectangular pen 14 by 11 mm (154 m^2) formed by chain link fence on three sides and on the fourth by solid fill. The pool was subdivided into three rectangles using booms[1] with floats projecting 15 cm above the surface and weighted skirts submerged to 30 cm. Each of the three rectangles formed by the booms measured 4.5 by 11 m (50 sq m) (Figure 3.1). The dolphins entered the test pool through a wire mesh gate into area #1. During the experiment, the test dolphins were able to interact visually and sonically with those in the adjacent pens.

A 5 m high observation tower was erected on the causeway 3 m from the north corner of the test pool. It provided a clear view of the entire study area. Video and sound recordings of all sessions were made from the platforms using an Hitachi GP-5U camera coupled to a Quasar VH5160SW recorder, a Uher 4200 stereophonic tape recorder, and a Sony BM 10 cassette recorder.

Acclimation

For 2 weeks, the animals were introduced to one loose oil boom placed in their holding pen. All three were then moved daily for 4 days to the test pool which contained several booms in various configurations. For 4 days thereafter, each dolphin was introduced alone into the test pool for up to 4 hours per day. During this time, the final boom configuration was established.

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[1] Oil Containment Systems Corp., Cocoa, FL.

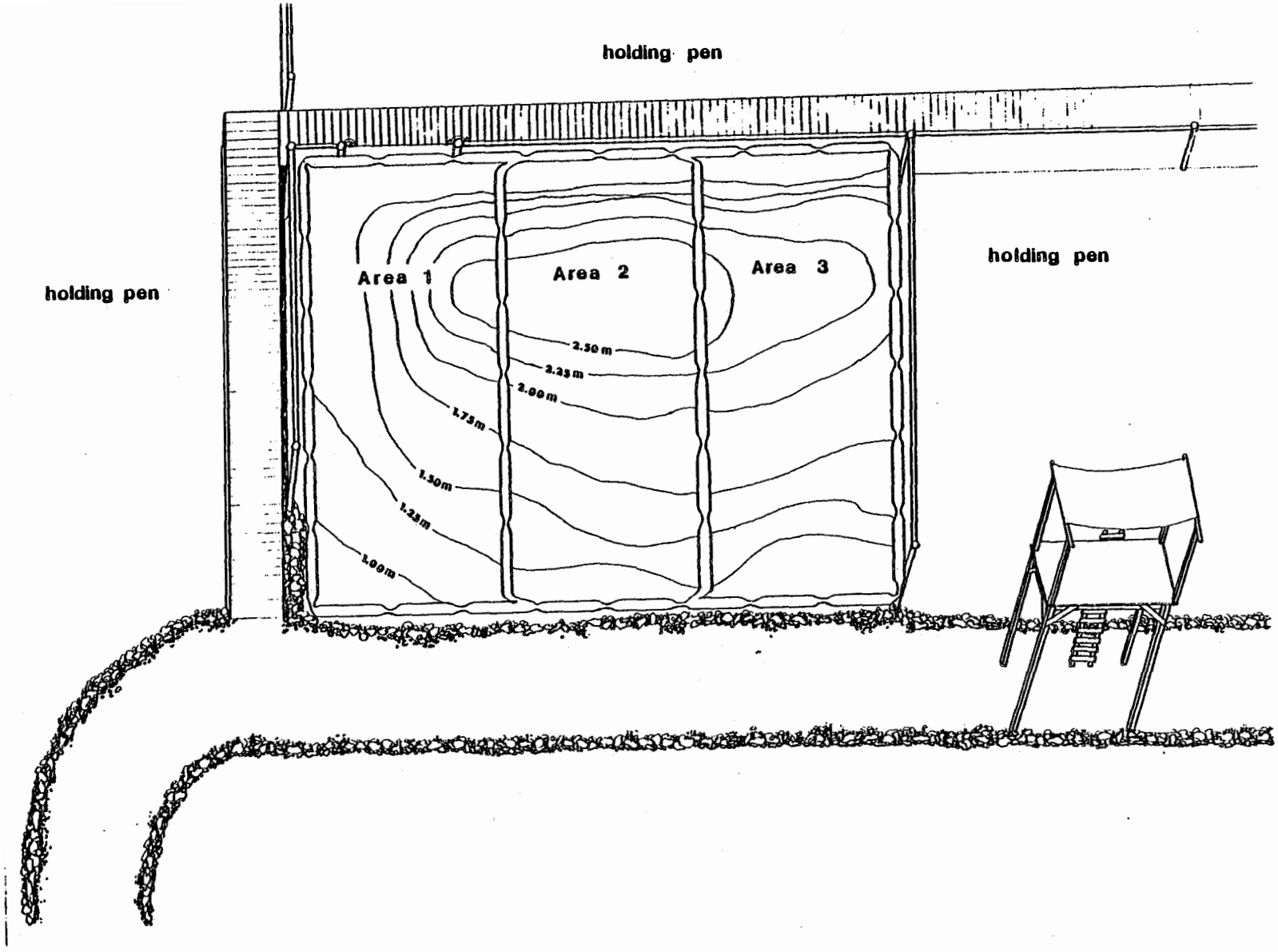

Figure 3.1 Test pool used in oil avoidance study, showing depth contours (at mean high tide), position of oil confining booms, relationship of adjacent dolphin holding pens, and location of observation tower. After a series of control sessions, colored mineral oil was added to Area 2.

Control Observations

During the control sessions without oil, each animal was put into the pool alone for 7 to 8 hours on four different days. Observations were made as soon as the animal entered the pen, and at 2 hour intervals thereafter. Each set of observations included a 30-minute period to note where the dolphin was surfacing, and a 15-minute "focal animal sample" (Altmann 1973), during which surfacings, underwater movements, dive duration and behavioral traits of the animal were recorded.

Test Observations

After control observations were made for 4 days on each dolphin, 416 L of oil was added to test area #2 to provide a surface slick approximately 1 cm thick. Each dolphin was then placed in the test pool on two separate days, and observations resumed on the same schedule.

We used Primol 355[2], an odorless, tasteless, non-toxic, highly refined mineral oil with a viscosity (67.8 cSt @ 40°C, Exxon Data Sheet DG-2H) resembling that of weathered Inter-Provincial crude oil (Geraci et al., unpublished data). Artist's oil paint colorant (Liquitex Series 1147, No. 244 Ivory Black[3]) containing bone black, aluminum stearate and linseed oil, was added to the Primol at a concentration of 0.5 g/L, to simulate the dark color of crude oil. A one-cm thick layer of the colored oil had an optical density of 0.7, clearly in the range of detectability (see Section 2.0, this report). Near the end of the third day of the oil session, the colorant had separated to some extent, forming dark aggregations. This was corrected by adding 100 litres of mineral oil containing 1.0 g/L of colorant. A luminescent sheen noted in both unoiled areas of the pool was

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[2] Exxon Company, P.O. Box 2180, Houston, TX 77001.
[3] Binney & Smith, Easton, PA 18042.

removed by absorbent pads[4] at the beginning of each day. These were also used to clean up the oil at the end of the experiment.

Analysis of Data

The experiment was designed so that each animal served as its own control. For each dolphin, we gathered data on frequency of surfacing in each pool area, interval between surfacings, and the frequency with which the animal would enter each area (occupation) between surfacings. These data were examined with respect to time of day (i.e. observation period) for the same animal under control (oil-free) and experimental (oil) conditions. Tests for pool area preference, diurnal and individual variation were made using a Friedman two-way analysis of variance by ranks (Siegle 1956).

Frequencies of underwater occupation of each pool area were derived from the maps of the movement of each dolphin during the 15-minute "focal animal sessions". An area was counted as occupied whenever an animal swam through it, between surfacings. The area where the animal dove was not included in that tabulation unless the dolphin left, then returned to that section of the pool, or resurfaced in the area without going into any other section of the pool.

The duration of dives were measured during 15-minute "focal animal sessions". They were grouped as means per observation period, and comparisons were made between dolphins, and between the oil and oil-free sessions. Means of dive times between the oil-free sessions and oil sessions were compared using Student's t-test.

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[4] Sorbent type 156 and 126; 3M Corp., St. Paul, MN 55144.

Results

The dolphins behaved normally throughout the acclimation period. In general their interactions were more conservative than when they were in their holding pen. When in the test pool alone, they swam slowly underwater, and regularly surfaced to breathe. There was little reaction to the booms, except by dolphin BLM-B which nudged the boom, occasionally dunking it with its snout. The presence of dolphins in adjacent holding pens did not obviously affect the test animals' behavior.

Throughout the acclimation and oil-free control periods, all three dolphins showed a preference for the portion of the pool with the deepest water (>1.75 m). With the booms in their final configuration, this represented test areas #2 and #3 (Figure 3.1) their preference for surfacing in these areas was significant for dolphins BLM-B and BLM-C ($X_r^2 = 6.50$; $P = 0.042$) and only slightly less so for dolphin BLM-A ($X_r^2 = 6.00$; $P = 0.069$). Similarly, the dolphins spent the greatest amount of time swimming underwater in test areas #2 and #3 respectively. This was significant for animals BLM-A and BLM-B ($X_r^2 = 6.5, 8.0$; $P < 0.05$ and 0.01 respectively), but not for BLM-C. There was no diurnal change in the surface frequency or dive times for any of the dolphins, but dolphin BLM-A moved less as the day progressed ($X_r^2 = 13.8$; $P < 0.01$).

During the control period, the dolphins avoided test area #1, surfacing in it less than 10% of the time, and occupying it only 18.3% of the time. We therefore chose to place the oil in one of the other areas - test area #2. When reintroduced into the pool, each dolphin clearly avoided the oiled area for 5, 35 and 53 minutes, respectively. Throughout the entire experiment, each dolphin came in contact with the oil from 2 to 7 times for a total of 12 contacts. These occurred within the first 2 hours of their

first exposure; none of the dolphins contacted oil on the second exposure, 4 days later. In all, the dolphins showed avoidance of the oiled test area #2. Before adding the oil, they had occupied this area 53.0% of the time and surfaced in it 51.0% of the time. After oiling, the frequency of occupation was dramatically reduced to 6.6%, and of surfacings to a mere 1.6%, making further statistical testing unnecessary.

On contacting the oil, the dolphins would respond in different ways. On three occasions, after merely touching the oil with the dorsal fins, they appeared to startle, suddenly increasing their speed and swimming into test area #3. Eight other times, they surfaced through the oil, but uncharacteristically did not breathe. Rather, they submerged abruptly, creating a splash, then moved quickly out from under the oil. After one episode, dolphin BLM-B returned to test area #3, blew a cloud of bubbles and slapped it's tail on the surface, whereas BLM-C, after swimming quickly into test area #3, released several loud exhalations in air and blew bubble clouds underwater.

No differences were found in the mean duration of dives among the daily observation periods for individual animals during the oil session. Comparison of dive times among the three dolphins showed minor differences. Dolphin BLM-B had a significantly shorter mean dive time in the oil session (19 sec.) than either dolphin BLM-A or BLM-C (22 and 21 sec. respectively; X_r^2 6.5; $P = 0.04$). When compared to their own dive times during the oil-free period (18, 17 and 17 sec. for BLM-A, B, and C, respectively) all dolphins exhibited longer dives when oil was present, but the difference was significant only for dolphin BLM-A ($t = 2.12$; $P < 0.05$).

Discussion

The dolphins in this study avoided oil. They clearly could detect it, since they swam under the oil, surfaced alongside in the clean water, but avoided contacting the oil itself. Their initial reaction was probably their normal response to a new stimulus, and does not necessarily imply a conscious avoidance to oil per se. After a short period of acclimation, each dolphin emerged in the oil, either accidentally or as part of an investigative process. All reacted immediately and overtly with a startle response followed by behavior indicative of stress or annoyance. After emerging two or three times during the first 1.5 hours of exposure, the dolphins did not again surface in the oil, either that day or when reintroduced to the pool four days later. This contact, though brief, was possibly a key stimulus reinforcing their complete avoidance of the oil. Yet the oil was innocuous, indicating that tactile perception alone was enough to evoke the behavior. This suggests that free-ranging dolphins and, perhaps, whales feeding and interacting in oil slicks (Goodale et al. 1979, Gruber 1980) do so, because they do not perceive the oil or are not disturbed by it, or because their motivation to escape is curbed by other factors. At sea these factors might include social interaction, pursuit by predators, harassment by intensive human activity such as might be associated with oil clean-up operations (Gill et al. 1967, Butler et al. 1974), or dependence on the area for food. Otherwise, visual and tactile perception leads to clear and effective avoidance of oil slicks by dolphins in an undisturbed setting.

References

(1) Altmann, J. 1973. Observational study of behavior: Sampling Methods. Behavior 49: 227-267.

(2) Butler, M.J.A., F. Berkes, and H. Powles. 1974. Biological aspects of oil pollution in the marine environment. A review. Manuscr. rep. 22A, 133 pp. Mar. Sci. Cent., McGill Univ., Montreal, Quebec.

(3) Gill, C., F. Booker, and T. Soper. 1967. The wreck of the Torey Canyon. David and Charles, Newton Abbot (Engl.), 128 pp.

(4) Goodale, D.R., M.A. Hyman, and H.E. Winn. 1981. Cetacean responses in association with the Regal Sword oil spill. In Cetacean and Turtle Assessment Programme, University of Rhode Island. Annual Report for 1979. U.S. Dept. of the Interior, WA. pp. XI-1 XI-6.

(5) Gruber, J.A. 1981. Ecology of the Atlantic bottlenosed dolphin (Tursiops truncatus) in the Pass Cavallo area of Matagorda Bay, TX. MS Thesis, Texas A&M University. 182 pp.

(6) Siegle, S. 1956. Non parametric statistics for the behavioral sciences. McGraw Hill, New York. 312 pp.

4.00 A STUDY TO DETERMINE IF GRAY WHALES DETECT OIL

From a Report Being Prepared by
Hubbs-Seaworld Research Institute
W. Evans, Director

Introduction

Laboratory studies at the Institute for Delphinid Research provided important data on visual and acoustic abilities of bottlenose dolphins to detect crude and refined oils. It may be possible to translate the findings from these studies to other members of the Delphinidae, and perhaps to other odontocetes, but not to mysticetes. Though mysticetes are presumed to have keen vision, there is no evidence that they echolocate. Their ability to detect oil, therefore, would rely more on vision and perhaps other sensory modalities; this would have to be established through independent studies. To determine whether baleen whales detect oil, a field study was undertaken by the Hubbs-Seaworld Research Institute, under the direction of Dr. W. Evans.

Gray whales, Eschrichtius robustus, annually make a 14,000 to 20,000 km round-trip migration between summer feeding grounds in the Beaufort, Chukchi and Bering seas, and winter calving/breeding lagoons off Baja California and Mexico (Pike 1962, Rice 1965, Rice and Wolman 1971). The whales typically follow the coastline within 10 km of shore until they reach Pt. Conception, California, where the majority deflect offshore. Between 5% and 20% of the whales continue along the coastal route (Dohl, Leatherwood, Woodhouse, unpublished observations). Extensive observations made on this highly visible and accessible species provide considerable background data on individual and group behaviors (Andrews 1914, Wyrick 1954, Leatherwood 1974,

This study was designed to assess the behavior of gray whales migrating in the presence of natural oil seeps emanating from the sea floor. Four land stations and one offshore drilling rig situated along a 50 km portion of the California coast were used as observatories from January 4 to January 23, 1981. Observers documented the swimming direction and speed, respiration rate and pattern, and the number of whales migrating through the study area, as well as the presence and extent of oil seeps in the water. Supporting behavioral data were obtained from a small aircraft and vessel.

Materials and Methods

The Study Area

Four shore observation sites, which were comparable in elevation and overlooked areas of similar bathymetry, were selected from Pt. Conception to Coal Oil Pt., California (Table 4.1, Figure 4.1). The eastern and western extremities of the study areas were fouled with oil, with at least four seeps within a 5 km radius of each observation site (Cal. Div. Oil and Gas Rep. TR08). The most active seep, near Coal Oil Point, releases a minimum of 30 barrels of oil per day. The intermediate observation areas at Sacate, Hondo and Refugio were considered "clean", although an oil seep has been documented in each area.

Observation Methods and Data Recording

Shore Stations

Eight observers were randomly assigned in pairs to the shore station; a single observer was stationed on the fixed oil rig Hondo. A senior observer at each site levelled and calibrated a surveyor's transit (Geotec T-24 or Leitz BT-20A) used for all sightings. Data were collected for groups of up to three whales migrating within a 5 km radius of the station. The

Table 4.1 Summary of Shore Station Characteristics

Site	Location	Comments	Observation Center Line	Inshore Distance	Range of Visibility	Depth 1 km	Range 5 km	Elevation	Observation Period
Point Conception	034°26.91'N 120°28.20'W	W of 202° mag Expected oil clean	202° mag	200 m	200°	40 m	110 m	64.4 m	Jan. 4-10 Jan. 12
Gacate	034°28.25'N 120°17.31'W	Expected oil free to oil light	163° mag	200 m	130°	15 m	90 m	70.5 m	Jan. 4-16
Platform Hondo	034°23.44'N 120°07.23'W	Expected oil free to oil light	267° mag	---	180°	50 m	250 m	41.9 m	Jan. 4-16
Refugio	034°27.78'N 120°04.38'W	Expected oil free	163° mag	500 m	150°	20 m	70 m	48.1 m	Jan. 11 Jan. 13-18 Jan. 20,21,23
Coal Oil Point	034°26.00'N 119°55.19'W	Expected oil dense	165° mag	200 m	150°	20 m	70 m	31.4 m	Jan. 4-16

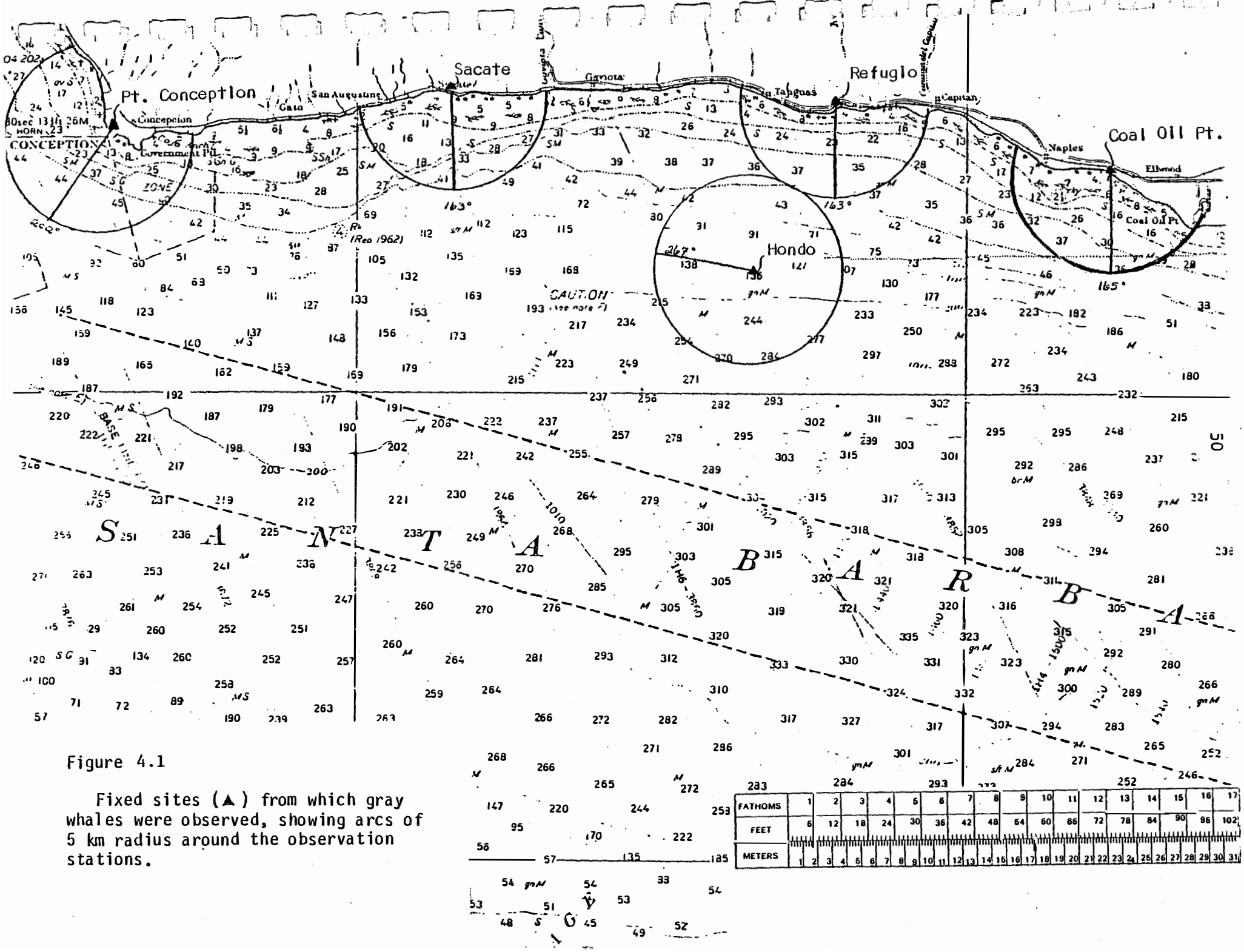

Figure 4.1

Fixed sites (▲) from which gray whales were observed, showing arcs of 5 km radius around the observation stations.

position of the whales was determined from a compass bearing (azimuth) and vertical angle (declination), and plotted on a bottom topography map. Swimming speeds were calculated from the time interval of sightings between fixed points. Water depth was derived from bathymetric charts. The frequency and number of blows were recorded for each set of sightings.

Vessel Surveys

Three observers on a small vessel monitored the whales' behavior by following small groups through the study area. Whales were approached from behind to minimize any disturbing effect of the vessel. One observer noted the number and behavior of whales, blows, presence of oil, and sea and weather states. A second observer correlated individual blow sightings with the position of the boat, thereby ensuring that the position fix recorded for the blow matched the actual location of the whales at the time the blow occurred. The third observer piloted the vessel and periodically reported magnetic heading and vessel speed. These data were relayed by intercom to a recorder, who also noted elapsed time and water depth.

Aerial Surveys

An experienced whale spotter determined location, direction of swimming, number of individuals and general behavior of migrating gray whales, both in the "coastal corridor" adjacent to the fixed observation points, and in the "offshore migratory corridor" outside the effective viewing range of the fixed points. Surveys were flown at 300 m in a Piper Super Cub modified for spotting fish. Coastal and offshore transects were flown according to the schedule outlined in Table 4.2.

For offshore surveys, one to four transects were randomly selected from a predetermined set of transects radiating from Pt. Conception at 10°

Table 4.2 Schedule of Aerial Surveys

Day	Date	Times of Day	Type of Transects
1	1/4/81	1112 - 1334	Coastal
2	1/5/81	0842 - 0901	Coastal (strip)
		0903 - 1103	Offshore - outbound 18 (172°) - inbound 20 (332°) - outbound 21 (142°) - inbound 22 (312°)
		1238 - 1354	Coastal
		1354 - 1430	Coastal (strip)
3	1/6/81	0844 - 1049	Coastal
		1052 - 1128	Offshore - outbound 23 (122°)
		1130 - 1141	Offshore - connecting 23 to 20 north end of Santa Rosa to north end of San Miguel
		1151 - 1209	Offshore - inbound 20 (332°)
4	1/7/81	0841 - 0938	Coastal
		0940 - 1110	Offshore - outbound 19 (162°) - inbound 24 (292°)
		1403 - 1530	Coastal
5	1/8/81	0842 - 1018	Coastal
6	1/9/81	1429 - 1531	Coastal
7	1/10/81	0917 - 1038	Offshore - outbound 22 (132°) - inbound 19 (342°)
8	1/11/81	0913 - 1024	Coastal
9	1/12/81	1053 - 1241	Coastal
10	1/13/81	1014 - 1132	Offshore - outbound 23 (122°) - inbound 21 (312°)
11	1/14/81	0849 - 1044	Coastal
12	1/15/81	1250 - 1413	Coastal
14	1/17/81	1030 - 1219	Coastal
15	1/18/81	0849 - 0937	Coastal
		0939 - 1035	Offshore - outbound 18 (172°) - inbound off-track
		1316 - 1515	Coastal
17	1/20/81	1300 - 1336	Coastal
20	1/23/81	0852 - 1031	Offshore - outbound off-track - inbound 20 (332°)
		1155 - 1300	Coastal

intervals (Figure 4.2). Coastal surveys were flown from Coal Oil Pt. westward to Pt. Conception along a series of fixed headings (Figure 4.3). Observations were logged on a tape recorder and the data were subsequently transferred to standard forms.

Monitoring Hydrocarbons

The aerial observer noted position and estimated extent of concentrations of oil on the surface. In order of increasing thickness, it was characterized visually as "silvery sheen", "heavy sheen","iridescent sheen", "dull gray or brown", "tar globs" "tar mat", and "thick oil", according to the terminology of Allen and Schleuter (1969).

Results and Discussion

Observations made during the three week field program as summarized in Tables 4.3 through 4.5. Each observation included data on group size, swimming speed and direction, pattern of respiration, water depth and distance from shore. Statistical tests are presently being used to compare these data for oil-free and oil-fouled areas. Since the data analysis is not yet complete, only preliminary statements can be made regarding the reaction of gray whales to the presence of oil on the surface of the water.

On several occasions aerial observers noted that gray whales, when approaching oil, changed their swimming direction. The change in behavior was not accompanied by any change in respiratory pattern or swimming speed, and in fact may not have been a response to oil. Typically, the whales would swim through oil, modifying their swimming speed (Table 4.6 and 4.7) but without a consistent pattern. Analyses of the respiratory behavior reveals some trends which are now being analyzed for significance. In oiled waters, the whales seem to spend less time at the surface, blowing less frequently but at a faster rate (Table 4.8).

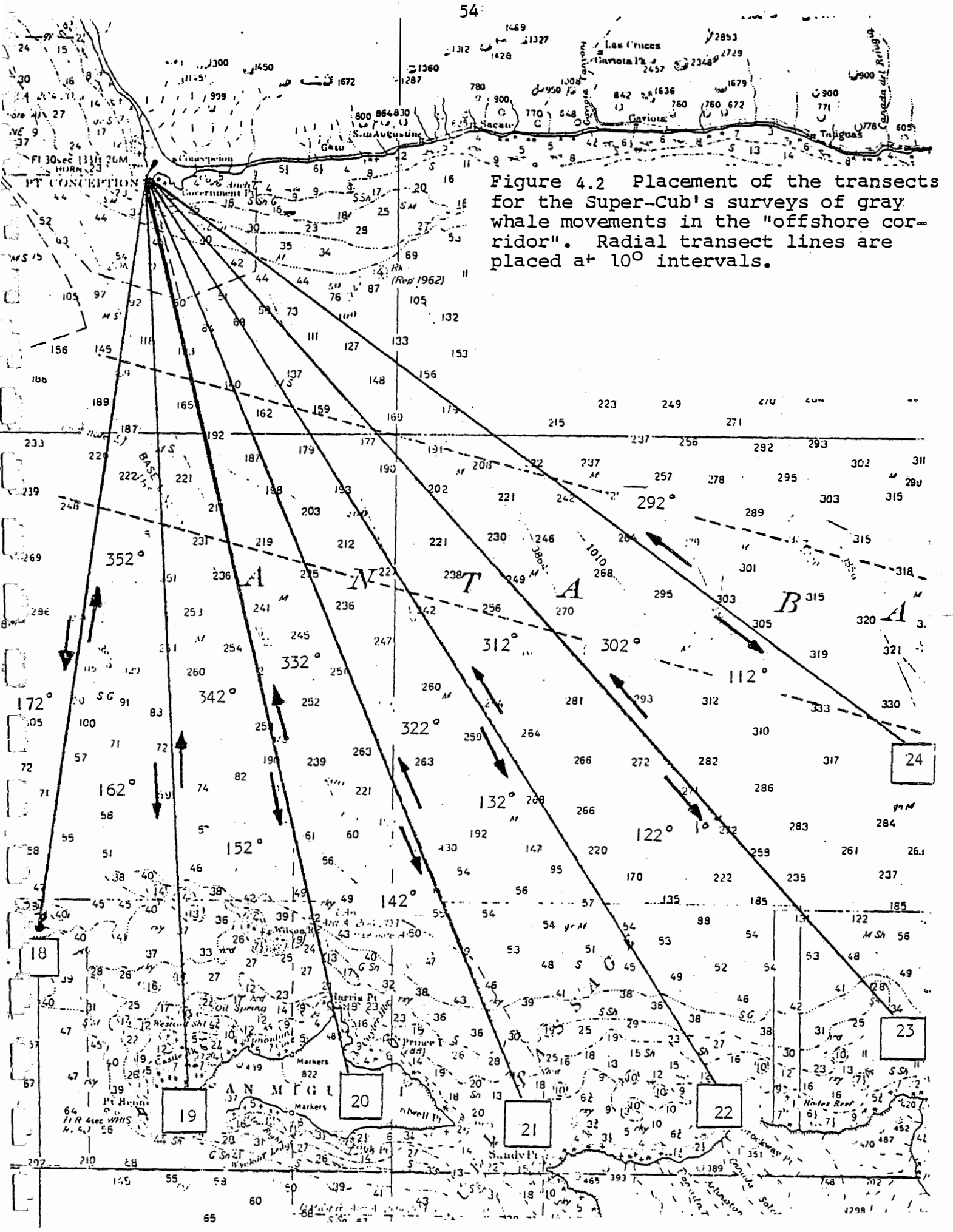

Figure 4.2 Placement of the transects for the Super-Cub's surveys of gray whale movements in the "offshore corridor". Radial transect lines are placed at 10° intervals.

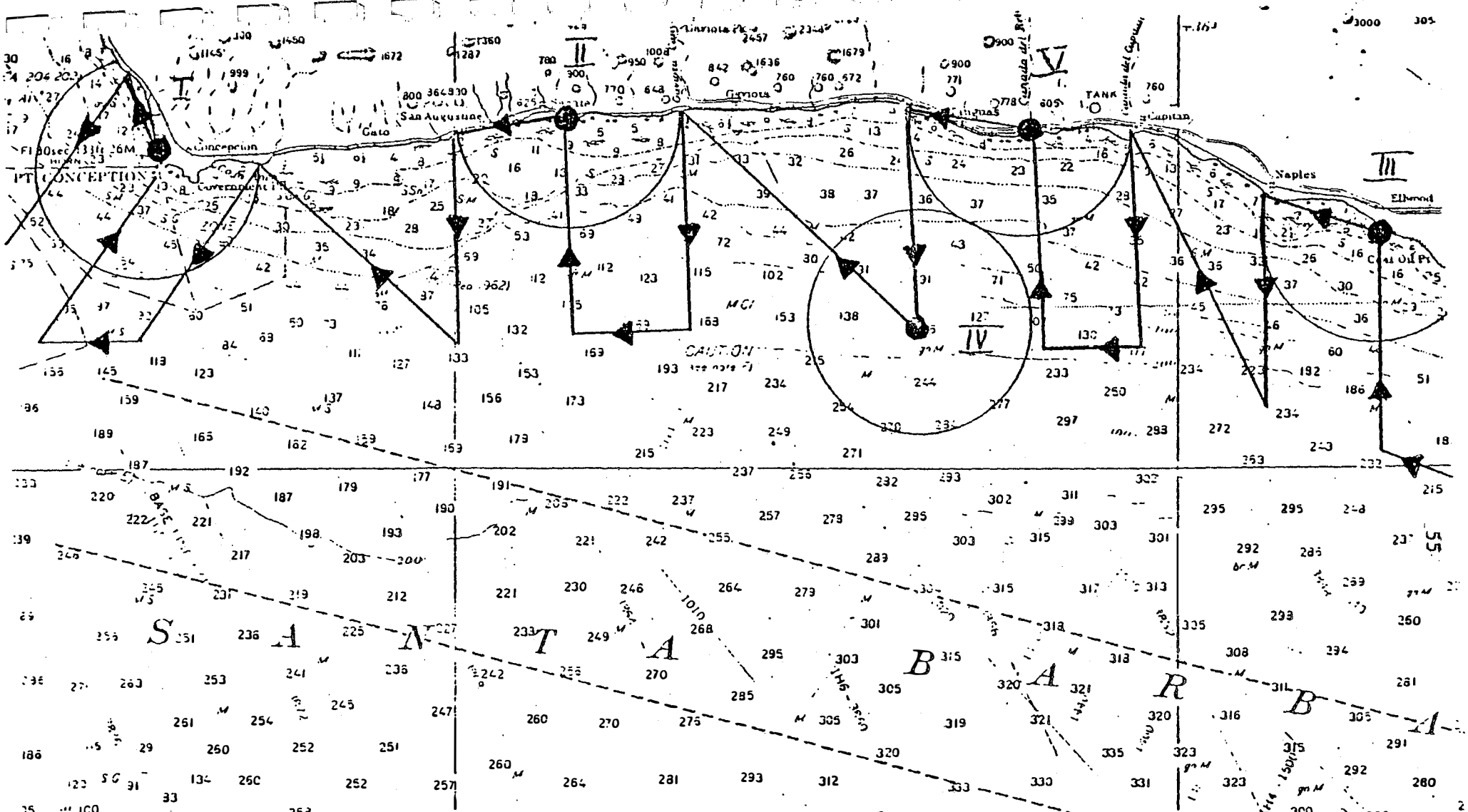

Figure 4.3

The fixed observation points, showing the "coastal" transects flown by the Super Cub during this study to note size of groups, position, and swim direction of gray whales.

Table 4.3 Summary of Observations for Week 1.

FIXED SITES:

Day	Trackings	Σ Whales Tracked	Σ Tracking Time	No. of Fixes	Untracked Whales	No. of Sightings
1	12	17	03:09'14"	55	5	132
2	25	61	10:08'19"	149	61	415
3	27	56	16:20'10"	403	63	882
4	21	42	14:37'43"	486	17	838
5	7	18	03:47'04"	50	20	260
6	14	37	10:27'04"	219	13	767
7	20	53	09:04'34"	154	29	390
TOTAL	126	284	67:34'08"	1516	208	3684

AIRCRAFT:

Day	No. of Flights	No. of Sightings	No. of Whales	Σ Time	No. of Island Flights	Cow/Calf Pairs
1	1	8	8	2:43		
2	5	18	64	5:28	3	
3	4	11	39	4:17	3	
4	7	37	95	5:45	3	4
5	1	1	1	2:16		
6	3	12	27	6:17		
7	5	27	98	3:33	3	1
TOTAL	26	114	332	30:19	12	5

VESSEL:

Day	Σ No. of Trackings	No. of Whales Tracked	No. of Fixes	No. of Sightings	Untracked Whales	Σ Tracking Time
1	2	4	18	98	2	1:22'14"
2	2	4	30	31	0	1:38'30"
3	2	3	157	159	5	5:17'54"
4	2	3	264	838	2	
5	Water Sampling					
6	1	3	68	146	1	4:50'19"
7	Propeller Lost - Out of Operation					
TOTAL	9	17	537	1272	10	18:39'44"

WEATHER: Mostly clear, with increased haze toward end of week

VISIBILITY: Mostly 3, then 2.

BEAUFORT: Predominantly 1, then 2.

Table 4.4 Summary of Observations for Week II.

FIXED SITES:

Day	Trackings	Σ Whales Tracked	Σ Tracking Time	No. of Fixes	Untracked Whales	No. of Sightings
8	6	12	02:38'54"	18	6	113
9	10	27	03:51'49"	81	3	211
10	6	12	03:57'10"	85	4	315
11	13	35	13:39'58"	226	7	546
12	7	11	02:52'59"	19	15	131
13	21	42	12:01'11"	191	0	
14			Change of Procedures			
TOTAL	63	139	39:02'01"	620	35	1316

AIRCRAFT:

Day	No. of Flights	No. of Sightings	No. of Whales	Σ Time	No. of Island Flights	Cow/Calf Pairs
8	2	23	43	3:19		3
9	1	5	23	2:52		
10	3	16	35	5:41		
11	2	15	31	5:01		
12	2	5	9	2:37		
13	1	11	18	2:03		
14	3	20	56	5:34		
TOTAL	14	100	215	27:07		3

VESSEL:

Day	Σ No. of Trackings	No. of Whales Tracked	No. of Fixes	No. of Sightings	Untracked Whales	Σ Tracking Time
8			Out of Operation			
9	1	10	38	82	0	00:45'55"
10	1	2	39	125	2	00:16'39"
11	2	6	90	207	0	05:16'27"
12			Water Sampling		8	
13	1	4	91	147	0	03:25'17"
14	2	7	80	234	4	03:46'48"
TOTAL	7	29	338	795	14	13:31'06"

WEATHER: Predominantly hazy, some rain on 12 I.

VISIBILITY: Mostly 3 and 2.

BEAUFORT: Mostly 1, then 2.

Table 4.5 Summary of Observations for Week III.

FIXED SITES:

Day	Trackings	Σ Whales Tracked	Σ Tracking Time	No. of Fixes	Untracked Whales	No. of Sightings
15	11	21	04:48'55"	43	1	371
16	Day off for boat, shore stations					
17	10	18	03:45'40"	33	7	134
18	5	8	00:52'50"	18	3	24
19	All rained out					
20	6	15	05:18'50"	42	5	132
TOTAL	32	62	14:46'15"	136	16	661

AIRCRAFT:

Day	No. of Flights	No. of Sightings	No. of Whales	Σ Time	No. of Island Flights	Cow/Calf Pairs
15	3	30	86	5:13	1	
16	3	12	20	2:02		
17	2	10	13	3:02		
18	3	8	17	4:36		1
19	Rained out					
20	2	11	21	4:04	1	
TOTAL	13	71	157	18:57	2	1

VESSEL:

Day	Σ No. of Trackings	No. of Whales Tracked	No. of Fixes	No. of Sightings	Untracked Whales	Σ Tracking Time
15	2	4	62	214	4	03:21'14"
16	Day off					
17	2	3	39	72	0	04:21'22"
18	1	2	57	121	5	02:32'39"
19	Rained Out					
20	Water Sampling					
TOTAL	5	9	158	407	9	10:15'15"

WEATHER: Mostly hazy and partly cloudy, with rain on 22 January.

VISIBILITY: Mostly 2 and 3.

BEAUFORT: Mostly 2, with 4 on 23 January.

Table 4.6 Swimming speeds of gray whales in relation to various types of oil films. Observations were made from a small vessel.

Type of Oil Film	No. Obs.	Swimming Speed (m/sec.)
none	713	2.13
small patches	14	2.30
continuous light sheen	39	1.80
tar globs	2	1.86
tar globs with oil	28	1.90
tar mat	11	1.75
thick oil	11	2.13

Table 4.7 Swimming speeds of gray whales in relation to intensity of oil seep activity. Observations were made from shore stations.

Oil Seep Activity	Station	No. Obs.	Swimming Speed (m/sec.)
none	Pt. Conception	349	1.65
none	Refugio	136	1.65
intermittent	Sacate	297	1.46
continuous	Coal Oil Pt.	325	1.43

Table 4.8 Respiratory patterns of gray whales in oil-free and oil-contaminated areas.

Respiratory Characteristics	Oil-free Areas	Areas with Oil Films	Significance
Time from first blow of one sequence to the first blow of the next sequence	272 sec.	288 sec.	$p = .360$
Average time between blows	32 sec.	26 sec.	$p < .001$
Time submerged between blow sequences	181 sec.	235 sec.	$p < .001$
Average number of blows in a surface interval	2.67 blows	1.91 blows	$p = .010$
Total time on the surface during blow sequence	91 sec.	55 sec.	$p = .001$

References

(1) Allen, A.A. and R.S. Schleuter. 1969. Estimates of surface pollution resulting from submarine oil seeps at Coal Oil Point and Platform A (California). Gen. Res. Corp., Santa Barbara, CA. Tech. Memo No. 1230. 43 pp.

(2) Andrews, R.C. 1914. Monograph of the Pacific Cetacea. I. The California gray whale (Rhachianectes glaucus. Cope). Mem. Amer. Mus. Nat. Hist. N.S. 1: 229-287.

(3) Leatherwood, J.S. 1974. Aerial observations of migrating gray whales, Eschrichtius robustus, off Southern California, 1969-72. In The California Gray Whale. pp. 45-49. W.E. Evans (Ed.). Mar. Fish. Rev. 36 (4).

(4) Pike, G.C. 1962. Migration and feeding of the gray whale (Eschrichtius gibbosus). J. Fish. Res. Bd. Can. 19: 815-838.

(5) Rice, D.W. 1965. Offshore southward migration of gray whales off southern California. J. Mamm. 46 (3): 500-501.

(6) Rice, D.W. and A.A. Wolman. 1971. The life history and ecology of the gray whale (Eschrichtius robustus). Amer. Soc. Mamm. Spec. Publ. No. 3: p. 142.

(7) Sumich, J.L. 1980. Swimming speeds, breathing rates, and costs of locomotion of migrating gray whales, Eschrichtius robustus. ms.

(8) Wyrick, R.F. 1954. Observations on the movements of the Pacific gray whale, Eschrichtius robustus (Cope). J. Mamm. 35: 596-598.

5.00 A RETROSPECTIVE ANALYSIS OF THREE TURSIOPS EXPOSED TO OIL ON THE SURFACE OF THE WATER

From a Report Prepared by
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Introduction

In 1968, a Florida marine mammal facility experienced an unprecedented and unexplained increase in mortality of its bottlenose dolphins. The only consistent clinical feature of the affected animals was elevated serum activity of glutamic pyruvic transaminese (GPT), suggesting liver damage. Intensive investigation by the staff members and consultants failed to identify the cause of the problem. However, the coincidental occurrence of an accidental oil spill from hydraulic equipment into the holding tanks led the investigators to suspect that exposure to the oil might have caused the mortalities.

To test this hypothesis, four Atlantic bottlenose dolphins were exposed to small amounts of oil applied over a 3 month period to the surface of the water in their holding tank. Blood samples were analyzed periodically during this period, with particular attention to SGPT. This experiment was performed in 1968, but the results have not previously been published.

Materials and Methods

Four adult bottlenose dolphins (Table 5.1) were captured off north-eastern Florida and acclimated for 3-6 weeks in a circular tank with a

Table 5.1 Basic data on experimental dolphins used in machine oil drip/ dump tests, all adults, at capture in northeastern Florida in 1968.

Animal Number	Standard Length (cm)	Sex	Date of Capture	Locality of Capture
243	230	F	August 1	ca. 2 km N Crescent Beach
244	234	F	August 1	ca. 2 km N Crescent Beach
248	242	M	August 13	off Crescent Beach
249	239	F	August 19	S of St. Augustine Beach

surface area of 33.7 m^2 containing filtered, copper-treated sea water at ambient ocean temperature. They were fed a mixed diet of 5.5 to 6.8 kg per day of fish, supplemented with multivitamins. The food fish variably included blue runner (Caranx crysos), butterfish (Peprilus triacanthus), thread herring (Opisthonema oglinum) and herring (Clupea harengus).

Beginning September 13, 1968, a mixture of equal parts Sinclair Duro Oil #250 and Standard Oil RMP Delco supercharge 3 SAE10 was dripped by burette onto the surface of the dolphins' tank, at a rate of 12 ml/day, five days per week. The oil formed a persistent visible sheen on the surface of the water. An additional 236 ml of the same mixture was poured onto the water surface at two week intervals. A total of 2716 ml of oil was applied from September 13 to December 20, 1968 (Table 5.2). On October 22, 1968, the dolphins were moved to a second tank with a surface area of 29.2 m^2. No adjustment was made in the amount of oil introduced into the pool to compensate for the difference in pool size.

Results

Two of the dolphins were pregnant when captured. This was not recognized until they gave birth; otherwise, they would have been removed from the study. Dolphin 243 bore a stillborn calf one month after the start of the experiment. Two days after the oil experiment was concluded, dolphin 249 bore a calf which survived almost two months.

The behavior and feeding activity of the dolphins was not affected by the presence of oil. The results of SGPT analyses (Table 5.3) did not suggest any destruction of liver tissue, except in dolphin 248. Pre-test levels of SGPT were somewhat higher in this dolphin than in the others, and more than doubled within two weeks of the start of the experiment. The activity of SGPT remained high in dolphin 248 throughout the experiment,

Table 5.2 Schedule for application of machine oil mixture to the surface of the water of the dolphin experimental exposure tank, with cumulative amounts for the total experiment. The smaller volumes in the center column are for drip; the larger values are for dump.

DATE (1968)	VOLUME (ml)	CUMULATIVE VOLUME (ml)
September 13	12 + 236	248
16	12	260
17	12	272
18	12	284
19	12	296
20	12	308
23	12	320
24	12	332
25	12	344
26	12 + 236	592
27	12	604
30	12	616
October 1	12	628
2	12	640
3	12	652
4	12	664
7	12	676
8	12	688
9	12	700
10	12 + 236	948
11	12	960
14	12	972
15	12	984
16	12	996
17	12	1008
18	12	1020
21	12	1032
22	12	1044
23	12	1056
24	12 + 236	1304
25	12	1316
28	12	1328
29	12	1340
30	12	1352
31	12	1364
November 1	12	1376
4	12	1388
5	12	1400
6	12	1412
7	12 + 236	1660
8	12	1672
11	12	1684
12	12	1696
13	12	1708

Table 5.2 cont'd.

DATE (1968)		VOLUME (ml)	CUMULATIVE VOLUME (ml)
November	14	12	1720
	15	12	1732
	18	12	1744
	19	12	1756
	20	12	1768
	21	12 + 236	2016
	22	12	2028
	25	12	2040
	26	12	2052
	27	12	2064
	28	12	2076
	29	12	2088
December	3	12 + 236	2336
	4	12	2348
	6	12	2360
	9	12	2372
	10	12	2384
	11	12	2396
	12	12	2408
	13	12	2420
	16	12	2432
	17	12	2444
	18	12	2456
	19	12 + 236	2704
	20	12	2716

Table 5.3 SGPT values for Atlantic bottlenose dolphins used in oil drip/dump experiments. The machine oil mixture was first applied to the surface of the water on September 13, 1968. Horizontal lines indicate values pre- and post-application of oil.

	DATE	ANIMAL NUMBER			
		243	244	248	249
pre-experimental	1 Aug. 1968	17	25		
	12 Aug. 1968	30	38		
	14 Aug. 1968			32	
	19 Aug. 1968				22
	26 Aug. 1968				29
	27 Aug. 1968	40	30	60	
	13 Sept. 1968	22	25	57	25
oil exposure	26 Sept. 1968	19	28	125	19
	30 Sept. 1968			110	
	16 Oct. 1968	17	19	130	23
	24 Oct. 1968	19	19	87	26
	31 Oct. 1968	29	34	126	22
	14 Nov. 1968	45	15	87	25
	26 Nov. 1968	50	43	83	41
	18 Dec. 1968	29	28	60	37

returning to pre-test levels at the end of the oil exposure period. No other clinical data are available to elucidate the nature of the liver dysfunction in this dolphin. Serum protein profiles were obtained on one occasion (Table 5.4) from 3 of the dolphins. Unfortunately dolphin 248 was not included.

No dolphins died and therefore no post mortem examinations were conducted as part of the study. Following the study, however, dolphins 248 and 249 were euthanized after 1 and 10 months respectively, and dolphin 243 died of a drug reaction, 2 months after the study had been terminated.

Post mortem examination revealed liver damage in all three (Appendix 5.1). Dolphin 243 was jaundiced and had evidence of liver necrosis and inflammation. Dolphin 248, which had an elevated SGPT during the experiment, had liver fibrosis, bile duct proliferation and chronic inflammatory cell infiltration. Dolphin 249 had similar liver pathology. The liver changes were characteristic of that associated with trematode parasites (Woodward et al. 1969) though no parasites were found.

Other post mortem findings of interest were stomach ulcerations in dolphin 243, and renal tubular necrosis in dolphin 248. The latter was associated with eosinophillic infiltration, suggesting that the lesion may have been induced by a parasite.

Discussion

Throughout the experiment, the dolphins had to surface through a persistent oil sheen, and were thus exposed to low concentrations of petroleum vapours, and contact particularly over the dorsal body surface. No skin or lung pathology was observed which could be attributable to the oil.

However, one dolphin had an elevated SGPT during the exposure period

Table 5.4 Serum protein profiles in 3 of 4 dolphins, after 2 months of exposure to small amounts of oil on the water surface.

	Dolphin		
	243	244	249
total protein (g/dl)	8.3	8.3	7.4
A/G	1.82	2.69	2.26
albumin (%)	64.7	72.9	69.2
gamma globulin (%)	19.6	14.7	13.5
other globulins (%)	15.7	12.4	17.3

This enzyme is located principally in the liver of bottlenose dolphins (Geraci and St. Aubin 1979) and its presence in serum reflects destruction of liver tissue. In this dolphin, SGPT reached levels which were approximately two to four times normal, compared with pre-experimental and published data (Ridgway 1972; Geraci and St. Aubin 1979). Combined clinical pathology and post mortem findings suggested acute and chronic liver damage the nature of which was more typical of a parasitic rather than a toxic insult. Nevertheless, the persistently elevated SGPT activity coincided with the onset and duration of exposure to oil, and we cannot discount the possibility that the two may be related.

The dolphins exposed to oil had poor reproductive success, but no worse than captive dolphins generally (Ridgway and Benirschke 1975). Dolphins conceived in the wild and born in captivity have a particularly poor record of survival, which is generally attributed to handling stress during capture and acclimation (D.K. Caldwell and M.C. Caldwell, pers. obs.). The calf mortalities cannot be clearly ascribed to the effects of oil on the pregnant females, but more likely were due to the frequent handling of the dolphins during the course of the experiment.

This study leaves many questions unanswered. Tissues of the exposed dolphins were not analyzed to determine if any petroleum fractions were assimilated or accumulated. Detailed study of the exposed skin would have been necessary to identify any surface contact effects. Blood analyses focussed on liver, in an attempt to duplicate the findings of the previous unexplained mortalities. However, more information regarding the effects of oil on dolphins might have been obtained had the scope of the blood analyses been greater. Despite these shortcomings, this unique experiment demonstrated that dolphins can tolerate extended exposure to small amounts of hydraulic oil, with no consistent deleterious effect, if any.

References

(1) Geraci, J.R., and D.J. St. Aubin. 1979. Tissue sources and diagnostic value of circulating enzymes in cetaceans. J. Fish. Res. Bd. Can. 36 (2): 158-163.

(2) Ridgway, S.H. 1972. Homeostasis in the aquatic environment. In Mammals of the Sea; Biology and Medicine. (Sam H. Ridgway, Ed.). Springfield, IL. Charles C. Thomas; 590-747.

(3) Ridgway, S.H. and K. Benirschke. 1977. Breeding dolphins: present status, suggestions for the future. Nat. Tech. Inf. Serv., PB-273 673.

(4) Woodard, J.C., S.G. Zam, D.K. Caldwell and M.C. Caldwell. 1969. Some parasitic diseases of dolphins. Path. Vet. 6: 257-272.

Appendix 5.1 Post mortem observations in bottlenosed dolphins exposed to oil on the water surface (presented as submitted).

History Dolphin 243

February 26, 1969 Received 22 grams Thiabendazole for stomach worms in A.M. Two hours after worming, seemed dizzy, loss of equilibrium, hit tank wall. Appeared to have recovered by 1300 hours. Did not eat in afternoon. Total food for day: 8 lbs (had eaten first thing in the morning to receive medication).

February 27, 1969 Ate only 6-7 lbs.

February 28, 1969 Found floating dead in tank at 1000 hours. Removed to cooler. Autopsy began at 1515 hours.

Gross Findings

Externally the animal was in apparently excellent condition. She appeared robust, and no external marks to note were seen except that there were a number of "tattoo marks" around the umbilical scar (four on one side, three on the other).

Muscle in apparently excellent robust condition. Blubber (especially more ventrally) showed more jaundice than we have seen in an adult for some time and there was considerable jaundice in the flipper joints and at the articulation of the skull with the vertebral column. No noticeable edema in connective tissues, but approximately one liter of yellowish clear fluid in abdominal cavity.

Respiratory system: Both lungs had a mottled appearance, but in general looked good. There were several small areas of mucus, and a few

areas indicating an old lungworm infection. No live worms found.

Circulatory system: Grossly normal in anatomy, but main vessels and chambers of heart showed evidence of more jaundice than usual. Weight of heart was 703.4 grams.

Digestive system: Gums did not appear to be in good condition, and apparently had been bleeding and particularly so on the lingual side. Teeth were in good condition, with the tips of some slightly worn. Several lesions around the teeth at the posterior end of each tooth row. No lesions on tongue. Esophagus grossly normal. Forestomach grossly normal, containing three undigested blue runner and two or three partially-digested blue runner in addition to a large number of fish bones. Condition of fish suggests animal had died shortly after feeding on 27 February or that digestive processes were markedly slowed. No lesions or parasites found in forestomach. Fundic stomach contained no food or parasites; several darkened areas on the stomach wall either bled very easily when touched or were already bleeding. Pyloric stomach grossly normal in anatomy, but coated with an almost black material that scraped off fairly easily; no parasites or lesions. Ampulla of duodenum somewhat reduced in size, but otherwise grossly normal with no lesions or parasites. Intestines with liquid bile-stained feces.

Liver: Yellowish white over entire surface, and internally very yellow. Hepato-pancreatic duct incised and no evidence of flukes found.

Spleen: Grossly normal in shape, but mottled blackish-whitish (granite-like) on surface. Weight 64.9 grams. Size normal.

Pancreas: Grossly normal.

Kidneys: Grossly normal.

Adrenals: Grossly normal. Left 13.0 grams, right 9.6 grams.

Urogenital System: Grossly normal in anatomy, with no parasites in bladder. Both ovaries had evidence of corpora. Fallopian tubes both had a good deal of discoloration. Post mortem urine was a deep green in color, and clear.

(Note: The clinical history of this animal and events that took place at the time suggested that she may have received an accidental overdose of Thiabendazole.)

Histopathology

Liver: The sinusoids connecting central veins are greatly dilated, although the liver is not severely congested. In the central portions of the liver, karyorrhectic nuclei can be observed, and many hepatocytes can be seen containing Councilman bodies or hyalin globules. Acute inflammatory cells can be seen within the sinusoids in the centrilobular area. The portal spaces are essentially normal, except the cells are swollen and show some degree of balooning degeneration.

Kidney: The kidney shows autolytic changes, but no significant lesions can be discerned.

Spleen: The spleen appears essentially normal. It is mildly congested and some degree of extramedullary hematopoiesis can be detected.

Heart: No significant lesions.

Duodenum: The lamina propria contains mononuclear inflammatory cells and one abscess can be observed.

Pyloric Stomach: No significant lesions.

Lungs: The lung shows a variable amount of interstitial fibrosis, pulmonary edema, congestion and old calcified parasitic nodules.

Adrenals: No significant lesions.

Pancreas: Minimal interstitial fibrosis is evident. The exocrine

pancreas is normal in appearance; however, Cowdry-type B intranuclear inclusions can be observed within the islets of Langerhans. There is no necrosis or evidence of inflammation in this area. Infiltrating the pancreas, a large splenic nodule can be observed. This organ is somewhat congested and contains hematopoietic elements.

Anatomical Diagnoses

1. Centrilobular dilitation of hepatic sinusoids with ballooning degeneration and necrosis.
2. Chronic interstitial fibrosis and pneumonia.

Dolphin 244

Dolphin died February 16, 1972 during parturition. No significant liver pathology observed on post mortem examination.

Dolphin 248

History

Euthanized on January 31, 1969.

Gross Findings

Externally the animal appeared to be in excellent condition, with abundant fat and no notable recent cuts or scrapes. Old deep cut in dorsal fin excised and found to have normal amount of scar tissue expected from an old cut, but no other evidence to indicate cause of injury. Two lesions on top of caudal peduncle which were open sores containing whale lice at capture, were completely healed with expected amount of scar tissue and pigment disturbance.

Muscle in good condition and no edema in the connective tissue, and no jaundice.

Respiratory System: Grossly normal. Some evidence of inactive calcified lungworms. Possible active worms as well.

Heart: Myocardium is essentially normal.

Stomach: Within the muscular tissue of the stomach, trematode parasites containing pigmentated ova can be observed. These are surrounded by dense sclerotic connective tissue which is infiltrated with numerous plasma cells, neutrophils as well as macrophages. Lymphoid aggregates are seen within the submucosa and infiltrate into the lamina propria to the surface epithelium. Numerous macrophages and other mononuclear cells can also be observed in the lamina propria.

Skin: Tissue sections taken from the area of cutaneous mite infection show erosion of the surface epithelium and acanthosis and pseudoepitheliomatous hyperplasia of the epidermis. Tissue sections taken from areas where white spots were observed show no significant lesions. There is no difference in the thickness of the epidermis in this area and differences in the amount of melanin pigment cannot be detected.

Anatomical Diagnoses

1. Hepatic and pancreatic trematodiasis, mild.
2. Chronic pneumonitis with pulmonary interstitial fibrosis.
3. Focal necrosis of renal tubules (suspected to be of parasitic origin due to presence of numerous eosinophils).
4. Gastric trematodiasis, Campulla gastrophilus
5. Cutaneous erosion and epidermal hyperplasia.

Dolphin 249

Euthanized on September 23, 1969, as part of studies unrelated to oil experiment. Postmortem examination did not reveal any significant liver pathology other than mild parasite-induced damage.

6.00 THE EFFECTS OF OIL ON CETACEAN SKIN

6.1 Morphological Assessment of the Effects of Oil on Cetacean Skin

Introduction

Sea otters and some pinnipeds whose hair becomes fouled with oil lose their ability to regulate body temperature (Kooyman et al. 1976, 1977, Williams 1978). This situation is not likely to occur in cetaceans as they are essentially hairless, and rely principally on blubber and vascular mechanisms for controlling body temperature.

Nevertheless, oil in contact with cetacean skin may have deleterious effects. Cetacean epidermis is a unique organ, having no counterpart in other mammals. It is morphologically peculiar in that it is composed of numerous tiers of viable cells. It is non-glandular and has no external keratinized layer. Prominent finger-like projections known as rete-pegs firmly anchor the epidermis into corresponding depressions in the underlying dermis. The anchor points are aligned symetrically in rows that sweep over the surface of the animal such that when swimming at high speed, prominent skin folds stand out in relief (Essapian 1955), possibly to reduce drag.

This basic hydrodynamic property would probably be jeopardized by anything that disrupts the physical and chemical characteristics of skin. Certain petroleum hydrocarbons damage the skin of terrestrial mammals (Dutton 1934, Villaume et al. 1976). In the present study, we sought to identify the manner and extent to which such compounds would affect the skin of cetaceans.

We approached the study in a number of ways. We determined the normal histology and ultrastructure of cetacean skin as a basis for understanding the dynamics of growth, healing, and the inflammatory response. We designed small cup-like discs which allowed us to place liquid hydrocarbons on precise areas of skin for controlled periods of time. We used bottlenose dolphins primarily, and other species as they became available, including a Risso's dolphin, Grampus griseus, and a sperm whale, Physeter catodon. We extended our comparative study to human subjects who voluntarily exposed their arms to small quantities of gasoline. The tests on humans, carried out under the same conditions as those on the dolphins, provided a frame of reference for assessing gross changes in cetacean skin.

After exposing the skin to crude oils and gasoline for up to 60 minutes, we determined the reaction by carefully observing the appearance of the contact site, and by using a thermographic procedure to detect increased heat radiation, as one indicator of inflammation. We undertook a histological and ultrastructural examination of biopsy samples of skin, for up to 7 days after exposure. In order to determine how petroleum might affect an animal with open wounds, we compared the progress of wound healing in experimental incisions, some of which were deliberately contaminated with oil. This sequence of studies has helped us to define the extent to which a realistic exposure to petroleum hydrocarbons affects the skin of toothed cetaceans.

Materials and Methods

Subjects Used for Surface Contact Studies

Three 3-year-old female dolphins, Tursiops truncatus, were used for the majority of skin contact studies. They were maintained in a 20 m x 10 m x 4 m deep sea water pen at the Institute for Delphinid Research

(IDR), Grassy Key, Florida. The dolphins had been in captivity for 6 months and were in good health, judging from physical condition and blood analyses. Each animal was fed twice daily, an average of 5.1 kg of frozen herring, Clupea harengus, providing 70 kcal per kg of body weight per day. Each dolphin was manoevered onto a standard dolphin transport stretcher that was then secured to a metal frame in the water. The dolphins were identified as BLM-A, BLM-B, and BLM-C. The sequence corresponded to the position of the animal's on the stretcher frame, and this order was maintained throughout the study (Figure 6.1:1).

A mature female Risso's dolphin, Grampus griseus, weighing 328 kg was made available for study by the New England Aquarium, Boston, MA. It was held in an indoor pool measuring 6.75 m x 10.5 m x 1.8 m deep. Water temperature was maintained at 17°-18°C, and salinity at 32 PPM; chlorine and pH were 0.05 PPM and 8 respectively. The dolphin ate 7.7 kg of herring per day providing 48 kcal per kg of body weight per day.

Seventeen persons, 8 males and 9 females, ranging from 22 to 43 years of age, made themselves available for one or two exposures to lead-free gasoline, for up to 28 minutes.

Construction and Application of Surface Contact Dishes

The surface contact (S.C.) dish used to expose both cetacean and human skin to petroleum hydrocarbons consisted of a 5 cm diameter round brass dish with a narrow lip and a 1.3 cm deep well[1] (Figure 6.1:2 A and B). Into the well was fitted a rubber ring[2] which is slightly thicker than

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[1] available as internal sliding door handle from hardware suppliers
[2] 1 1/4" regular basin P.O. Plug Gasket; Unit #303, Master Plumber Products Ltd., Hamilton, Ontario

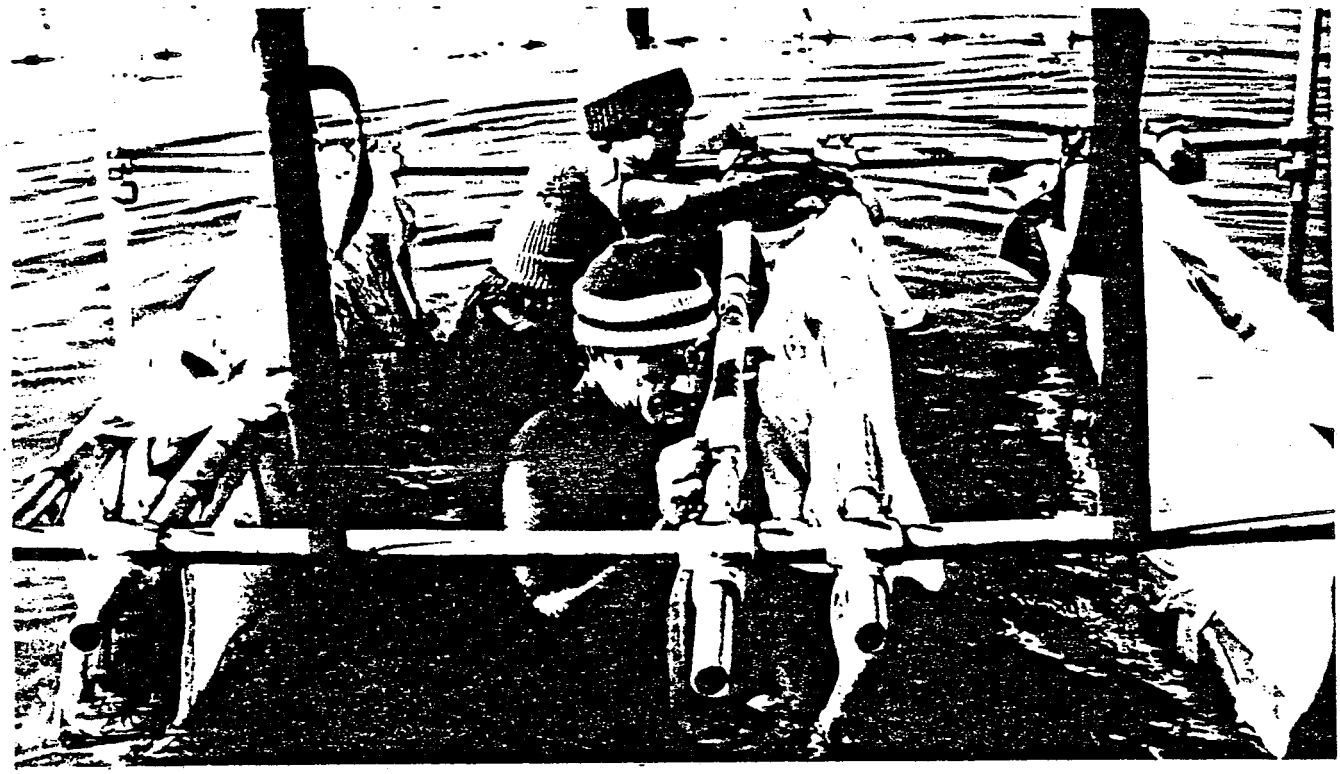

Figure 6.1:1 Three Tursiops used in the surface contact studies at IDR. Each animal is placed in a standard dolphin transport stretcher and secured to a metal frame in the water so that the body is partially submersed exposing only the back and blowhole. A muslin sheet is draped over the back of each dolphin, protecting the skin from drying and exposure.

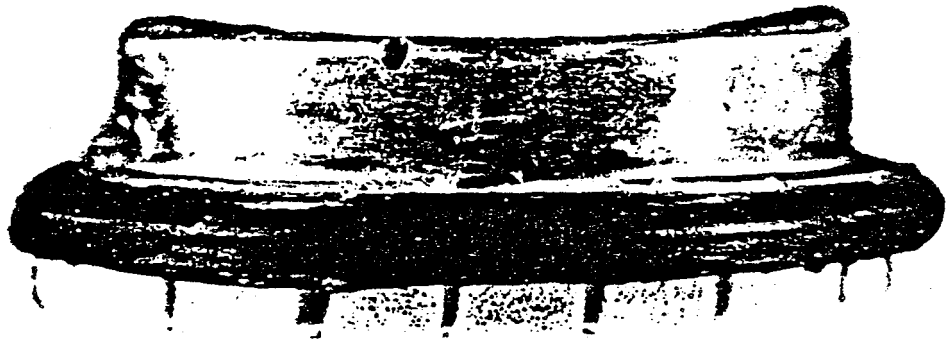

Figure 6.1:2 A: Side view of the surface contact dish. The lower (contact) surface of the appliance consists of a gasket of PVC weather stripping mounted on a rubber seal fitted into the brass dish. The appliance measures 5 cm in diameter, and has a 1.3 cm deep well.

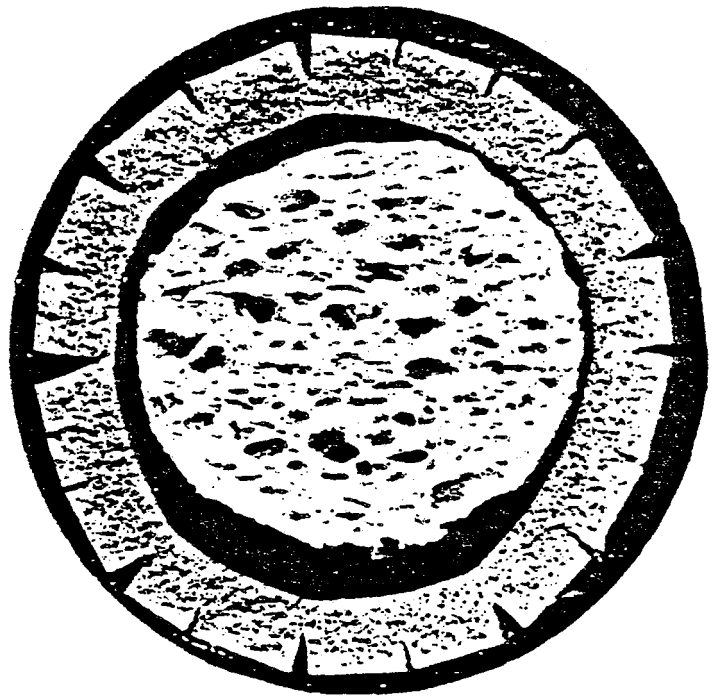

Figure 6.1:2 B: Bottom view of the surface contact dish showing a 3 cm wide disc of cellulose sponge inserted into the brass dish, and surrounded by the PVC gasketing material.

the depth of the well so that after it was secured with silicone sealant, a ring of rubber protruded slightly below the lip of the dish. A strip of adhesive-backed closed-cell PVC,[3] 0.8 cm wide was then bonded with silicone sealant onto the rubber rim, forming a gasket (Figure 6.1:2 A and B).

Cellulose sponge was used as the absorbing medium to retain the fluid in the S.C. dish. The sponge was cut into discs approximately 3.0 cm in diameter and 1.5 cm thick. Each was friction-fitted into a dish well leaving 0.3 to 0.6 cm of sponge protruding. This was done to ensure contact between the sponge and the skin.

In operation, a number of sponge-discs were placed in a 200 ml sealable polyethylene container filled with 100 to 150 ml of test petroleum product, at least 30 minutes before each contact trial. Once saturated, each disc was placed into an S.C. dish well. The prepared units were then stored in a sealed rectangular polyethylene container to minimize evaporation and contamination.

The intended contact area of the skin was wiped dry with a 10 cm x 10 cm 12 ply gauze pad and up to four S.C. dishes were placed and held securely on the prepared skin surface, using only slight finger pressure (Figure 6.1:3 A and B). The dishes were placed in the same general region on the back of the animals, just forward of the dorsal fin. This area provided a smooth, horizontal surface with ample room for placing 9 or more dishes for as long as 75 minutes. On human subjects, a single S.C. dish was placed on the inner, usually hair-free surface of the forearm, one-third of the way down from the elbow. The person held the dish in place with his or her free hand. As a measure of safety, we always

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[3] Myro door seal. Myro Inc., Milwaukee, WI 53209

Figure 6.1:3 A Two surface contact dishes containing sponges saturated with test material are placed on the prepared skin surface on the back of a Tursiops, just cranial to the dorsal fin.

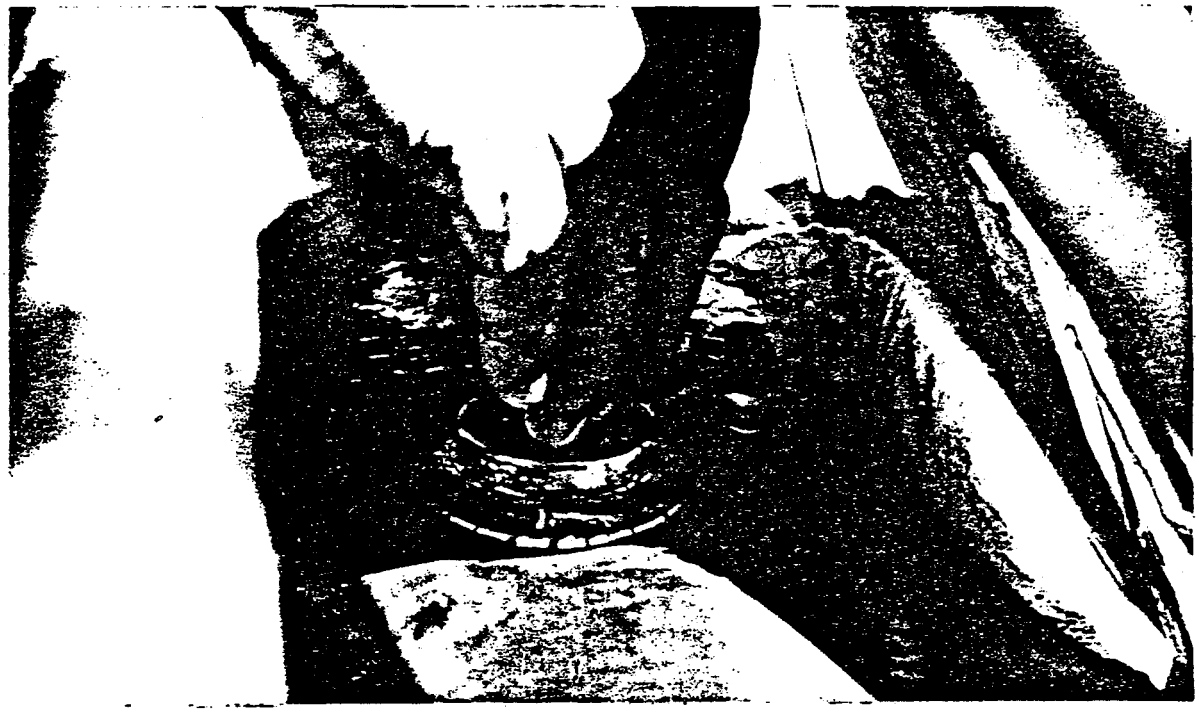

Figure 6.1:3 B An attendant applies and maintains slight finger pressure on both surface contact dishes.

applied dishes to left arm of right-handed individuals, and vice versa.

Immediately after use, the sponge was removed from the S.C. dish and discarded. The dish was placed in a container of water with Pine Sol detergent[4] for 2-3 hours, then transferred to a second container of water and detergent, scrubbed, rinsed with clean water, and allowed to air dry. Once dry, the dishes were stored in sealed, polyethylene containers for re-use.

Recording Grossly Observable Changes Associated with Surface Contact

A complete description of the location and appearance of the skin area exposed to oil was recorded. This included color, shape, surface configuration, and integrity of the exposed and surrounding skin. Tabular and photographic records, and sketches were maintained on a daily basis initially, and at longer intervals thereafter, until the contact areas were visually indistinguishable from non-exposed skin.

The human subjects also verbally recorded sensations associated with the exposure, noting the type of sensitivity (heat, pain, pressure), and the extent of the area involved. When people were tested as a group, they were instructed not to respond verbally during the trial.

For a select number of trials on dolphins and humans, the pattern of heat radiating from the contact sites and surrounding tissues was monitored using an AGA Thermovision, Model 680 Medical[5]. A photographic record of the heat patterns was maintained during the exposure on the dolphins, and immediately following exposure on both the dolphins and the humans, using a Tektronix Oscilloscope Camera System, Model C-27[6]. Actual temperature

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[4] Cyanamid, Toronto, Ontario, Canada
[5] AGA Infrared Systems, AB, S-181 81 Lidingo, Sweden
[6] S.W. Millikan Way, P.O. Box 500, Beaverton, OR 97005

measurements were made using an AGA Temperature Controller TC-2[7] as a reference. The controller was set at 28°C and placed in the area to be photographed. The color of the controller disc could then be matched with the corresponding color bar at the base of the photograph. For comparative purposes, we injected 0.1 ml of turpentine, a known irritant, into the epidermis of a dolphin, and monitored the vascular response using thermography.

Obtaining and Processing Skin Samples for Histology and Ultrastructure

Skin samples from live cetaceans were removed in the form of wedge biopsies, using a straight-edge No. 10 scalpel blade. The biopsies measured approximately 10 mm x 4 mm and penetrated the dermal layer. All specimens were placed in a petri dish and bisected longitudinally. One half, for electron microscopy, was diced into cubes approximately 1 mm x 1 mm x 1 mm and transferred to 4 ml of cold 2% gluteraldehyde (0.1 M Na cacodylate buffer). The other half, for light microscopy, was placed in 2 ml of 10% neutrally buffered formalin. Samples were kept on ice until they could be stored at 4°C.

The gluteraldehyde-fixed material was washed in 0.1 M Na cacodylate buffer, postfixed in 1% osmium tetroxide in 0.1 M Na cacodylate buffer for 4 hours at 4°C, dehydrated in acetone, and embedded in epon. These tissues were sectioned with glass knives in a Reichert[8] OmU_2 ultramicrotome. One micron sections were stained with 1% toluidine blue in a 1% sodium borate solution at 90°C. Thin sections were stained with uranyl acetate and lead citrate and examined in a Hitachi[9] HS-9 electron microscope with an accelerating voltage of 70 KV.

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[7] AGA Infrared Systems, AB, S-181 81 Lidingo, Sweden
[8] Sargent-Welch Scientific of Canada, Ltd., Toronto, Ontario, Canada
[9] Hitachi Scientific Instruments, Nissei Sangyo Canada, Inc., Rexdale, Ontario, Canada

Assessing the Effects of Petroleum Hydrocarbons on Wound Healing

We approached this segment of the study in two ways. We observed gross and histological changes associated with the healing process of the more than 200 biopsy wounds from exposed and non-exposed skin of the Risso's and bottlenose dolphins that had been the principal subjects of the surface contact study. We recorded the progress of wound healing, noting color, integrity, shape, size, the appearances of the cut surface, and the apposition of the edges.

In a more direct approach, using bottlenose dolphins, we compared the progress of wound healing in experimental skin incisions, some of which were deliberately contaminated with oil. To accomplish this, we made two parallel 10-15 cm long, incisions transversely across the back of Tursiops BLM-B and BLM-C, using a straight-edged No. 10 scalpel blade. The incisions were made 5.5 cm apart, approximately 25 cm cranial to the dorsal fin, and only deep enough to barely expose the blubber layer. The incision closest to the dorsal fin was reserved as a control. West Texas crude oil was gently massaged into the other wound for 60 and 30 minutes, respectively, in BLM-B and BLM-C. The entire area was subsequently rinsed with sea water. During the infusion of the wounds on BLM-B, thermographic records were made to monitor changes in skin temperature.

Biopsy samples were removed at right angles to and along the course of the incisions, at 10 and 30 minutes, 1, 2, 4, 8, 24 hours, and 2, 3, 4, 5, 6, and 7 days after exposure to oil. Each specimen was placed in a petri dish and stored on ice while hemostatic powder[10] was applied to the wound. The sample was then bisected longitudinally, one half prepared for electron microscopy, the other for light microscopy.

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[10] Haver-Lockhart, Mississauga, Ontario, Canada

Results and Discussion

Subjects

The bottlenose dolphins were ideally suited as the principal subjects of the surface contact studies. They required a minimum number of handlers, and each animal was subjected to no more manipulation than was needed to guide it onto a stretcher. During 3 blocks of trials, the dolphins were each captured up to 10 times weekly for a total of 74 times. Each dolphin remained on the stretcher until work was completed on all three, usually within 4 hours, but on occasion, for up to 6 hours. The dolphins remained calm, and sustained no injuries associated with the capture and retention. All ate food offered within 5 minutes after release from the stretcher. In all, the operation was simple, safe, and effective.

Surface Contact Dishes

The S.C. device fulfilled all of the criteria for applying petroleum hydrocarbons to the skin of cetaceans. The parts were commercially available, did not react with petroleum products, were not irritating to epidermis, and were easy to assemble. The device was easily cleaned and was re-usable. The dish design enabled controlled contact of a test product with the epidermis, while minimizing leakage and evaporation of the compound. Because of their small size, several S.C. dishes could be placed on the same animal, thus allowing a number of individual exposures to be made simultaneously.

Grossly Observable Effects of Crude Oil and Gasoline on Cetacean and Human Skin

West Texas crude oil was applied for 25 minutes to the skin of BLM-A and BLM-B. Inter-Provincial crude oil was applied for 45 minutes to BLM-A only. All three Tursiops, and the Risso's dolphin were also exposed to lead-free gasoline for periods variably ranging from 15 min. to 75 min. Immediately following the application, and quite independent of the type of product or duration of exposure, some of the contact sites were uniformly pale against the background of natural color; others were apparently unaffected (Table 6.1:1). In some cases, the exposed skin had a faint hobnail texture that disappeared within 5 minutes. Normal color was always restored within 2 hours. At no time was there any swelling, hemorrhage, or break in the continuity of the skin associated with exposure.

In marked contrast, human skin in contact with lead-free gasoline for up to 35 minutes became distinctly red over the entire contact site. Normal skin coloration was restored within 10 days in most subjects, while the discoloration persisted after 7 months in another (Table 6.1:2 and 3, Figure 6.1:4). This variation is consistent with other observations on human beings (Anderson 1953, Lupulescu et al. 1973).

Results of the infrared monitoring showed mild differences in the surface temperature between dolphin skin exposed to lead-free gasoline, and saline. The skin exposed to gasoline 1°C warmer than the saline-exposed area. Generally the skin surface within a 10-15 cm radius of the contact sites was 1° to 2°C cooler than the more distant unexposed skin, a probable result of evaporative cooling. No discrete vascular reaction associated with gasoline could be identified thermographically (Figure 6.1:5 A and B). On the other hand, human skin exposed to lead-free gasoline between 10 and 20 minutes showed discrete areas of heat dissipation at the contact site immediately after the dishes were removed, and in one subject, for up to 48

Table 6.1: Appearance of skin after removal of surface dishes containing petroleum hydrocarbons

Exposure time (min)	W-T Crude Oil 25	I-P Crude Oil 45	Lead-free Gasoline 15	30	45	60	75
Species Tursiops truncatus BIM-A	-an area of pale gray on each dish site; normal color returned in 10 min.	-an area of pale gray on each dish site; normal color returned in 10 min.	-smooth; glossy; pale gray; slightly raised area beneath dish site; normal appearance returned in 75 min.	-pale gray area extends beyond contact site; mottled gray contact site -[1]small blisters under 1 dish; normal color returned in 5 min.	-no visible reaction	-no visible reaction	--
BIM-B	-an area of pale gray on each dish site; normal color returned in 10 min.	--	-an area of pale gray on each dish site; wrinkled contact area; slightly raised surface; mottled gray halo of ∿ 10 cm diameter -normal color and texture returned in 30 min.	-one dish mark, slightly paler gray than surrounding tissue; normal coloration returned in 30 min.	-an area of pale gray on each dish site; slightly wrinkled; small blisters on dish areas; normal color and texture returned in 5 min.	--	-no visible reaction
BIM-C	--	--	-an area of pale gray on each dish site; normal color returned in 2 hours	-an area of pale gray on each dish site; small blisters on contact areas; normal color returned in 6 days	--	-an area of pale gray on each dish site; slightly puckered and raised; normal color returned in 3 min.	-no visible reaction
Grampus griseus	--	--	--	--	-an area of pale gray on each dish site; normal color returned in 5 min.	--	--

[1] Disseminated small blisters

Table 6.1:2 Appearance of representative contact sites on human skin after removal of surface dishes containing petroleum hydrocarbons

Subject	Petroleum product	Exposure time (min.)	Time after application		
			0	10 days	3 months
1 female 22 yrs.	Lead-free gasoline	15	-discrete red circular area on contact site, surrounded by pale pink halo∿ 5 cm wide -mottled discoloration; from wrist to elbow; contact area slightly raised	-uniformly circular red-tan discoloration on contact site; skin surface had a dry, scaly texture	-no mark
2 male 31 yrs.	Lead-free gasoline	15	-discrete red circular area on contact site; mottled pink halo ∿ 3 cm wide around red area	-dark brown-red circular mark on contact site	-brown, circular mark on contact site
3 male 43 yrs.	Lead-free gasoline	15	-slightly pinkish area on contact site; the discoloration disappeared within a few hours	-no mark	-no mark

Table 6.1:3 Summary of the reaction of human skin to topical application of lead-free gasoline using a S.C. dish for 8-35 min. Observations are based on 20 contacts in 17 individuals (9F; 8M).

Time After Exposure					
Immediate		10 Days		3-7 Months	
discrete, red circle	10	discrete red-tan circle	4	discrete brown circle	2[a]
pale red circle	3	dry, scaly	12	no mark	18
pink halo around dish mark	2	blisters	2		
general mottling on forearm	1	dark brown halo	2		
indiscrete red area	2	discrete pink circle	5		
indiscrete pink area	3	no mark	8		
white, discrete area	1				
mottled halo	1				

[a] both contact sites were on one individual

Figure 6.1:4 A

Contact area on human volunteer 5 min. after removal of disc containing lead-free gasoline (35 min. exposure). The exposed skin was wrinkled, and pale, and was surrounded by a reddish halo.

Figure 6.1:4 B

Contact area 3 days after 15 min. exposure to lead-free gasoline. The skin is dry with a scaly texture, and dark-tan discoloration.

Figure 6.1:4 C

Same subject as in Figure 6.1:4 B, 8 days after exposure. The surface had blistered and sloughed the superficial epithelium. A brown discoloration (similar to that shown in Figure 6.1:4 B) persisted in this individual for over 7 months.

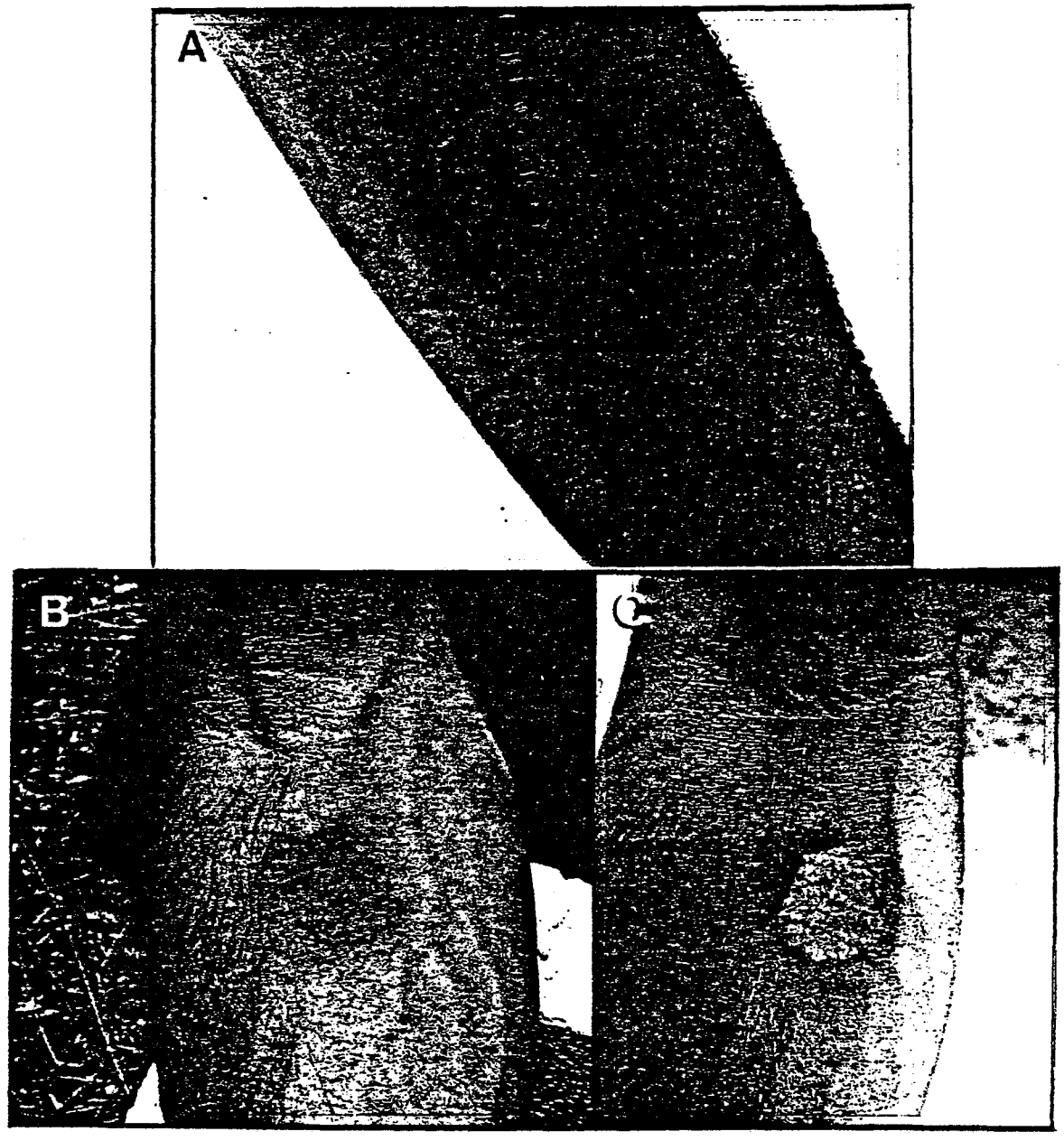


Figure 6.1:4 A - C

Figure 6.1:5 A

Oil dishes in place on the dorsal surface of a bottlenose dolphin. The dish on the right side contained lead-free gasoline; that on the left, sea water as a control. Reference probe (disc with central white dot) was set at 28°C. The corresponding color is indicated on the color series on base of the plate. Each color represents a 1°C increment from cool on the left to warm on the right. In the exposure the blue on the hand of the attendant is the source of greatest heat radiation (32°C or greater).

Figure 6.1:5 B

Oil dishes were removed after 15 minutes. (Reference probe is set at 28°C.) The temperature of the area under the gasoline dish (finger on right) is 26°C, whereas that exposed to sea water (finger on left) is 25°C, and the entire area is 1°-2°C cooler than unexposed skin distant from the contact sites.

Figure 6.1:5 C

Arms of human subject #1 (Table 6.1:2) following exposure for 15 min. to lead-free gasoline. (Reference probe set at 28°C.) The contact site on the arm on the right is evident as a diffuse area of 34°C with a core of 35°C or greater. The surrounding skin is 1° to 2°C warmer than the corresponding area on the non-exposed arm.

Figure 6.1:5 D

Arms of human subject #3 (Table 6.1:2) following exposure for 15 min. to lead-free gasoline. (Reference probe set at 28°C.) The contact arm on the right shows the same broad pattern of increased heat radiation from the disc site.

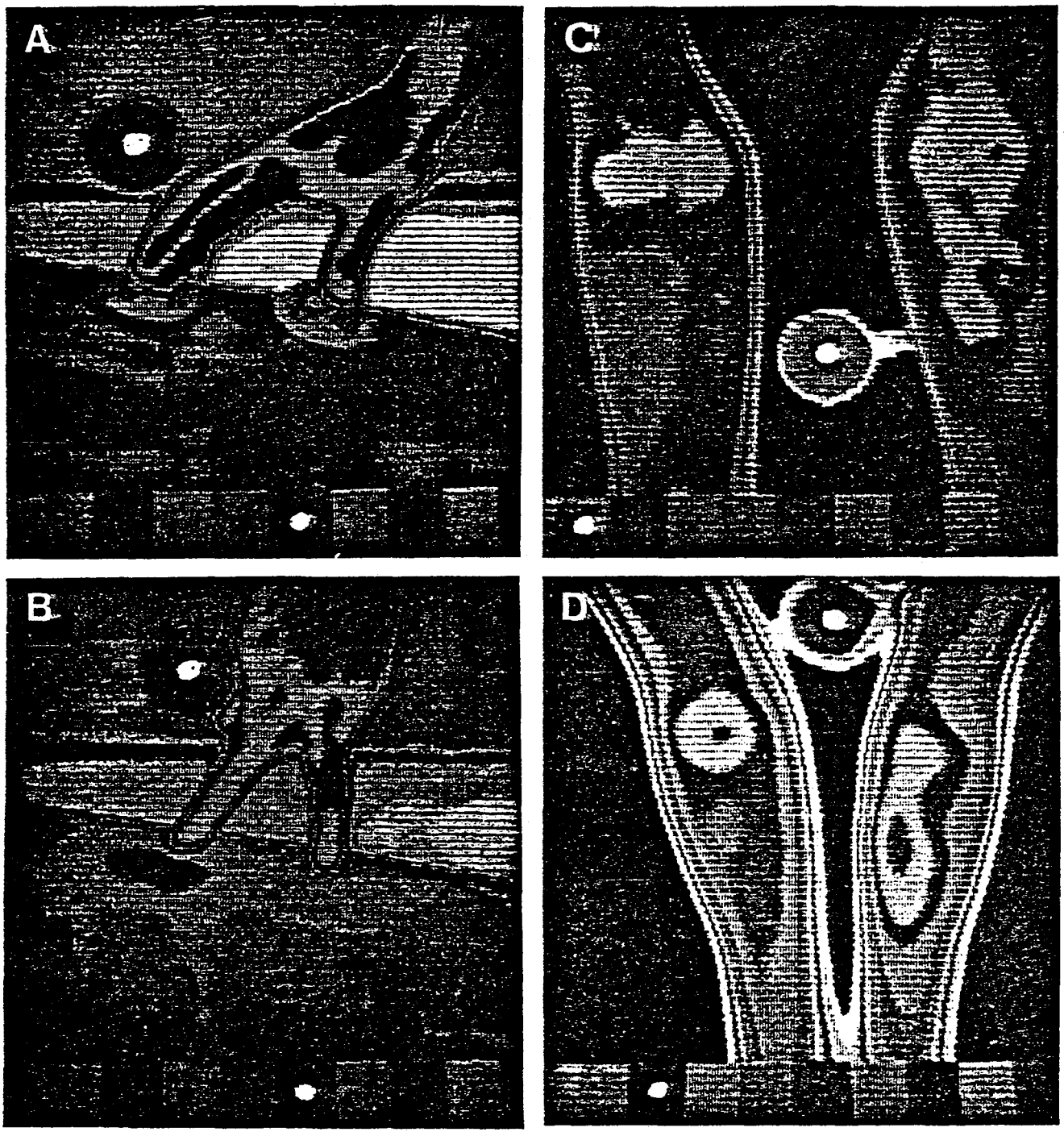


Figure 6.1:5 A-D

hours later (Figure 6.1:5 C and D). Large areas of the exposed forearm generally could be 2°-3°C higher than the corresponding area on the control arm.

All of the subjects reported a tingling, burning sensation after 10 minutes of exposure. The degree of perceived burning increased until the persons elected to remove the dishes. In 3 individuals, the skin at the contact site peeled several days after the exposure. Most other persons reported a superficial drying and flaking of the contact area.

The skin reaction to gasoline in the human subjects was more severe, or at least more apparent than it was in dolphins. Inflammatory changes were more visible in humans, in which the intense reddening associated with acute inflammation is easily distinguished from flesh tones. In dolphins only marked changes in gray tone were observed. The classical signs associated with the inflammatory response which we observed in response to injection of turpentine, a known irritant (Figure 6.1:5 E and F), were not observed in dolphins exposed to crude oil.

Histology of Cetacean Skin

The literature on cetacean epidermis is confusing. A variety of names have been used to describe the same structures. Earlier observations appear to be inaccurate in light of subsequent studies and some interpretations of the ultrastructurally observed cell components appear to be incorrect. Before describing the changes in cellular morphology associated with oil, we will attempt to clarify some of these discrepancies and present a summary description of the normal epidermis of Tursiops truncatus.

The greatest confusion in the literature arises from a controversy over the number of cellular strata present in cetacean epidermis and their

Figure 6.1:5 E

Infrared pattern associated with a known irritant (0.1 ml turpentine) injected intradermally into 8 locations along the back of a bottlenose dolphin. After 32 minutes, each injection site is marked by a core of 34° to 35°, surrounded by concentric zones of decreasing temperatures, against a skin temperature background of 29° to 30°C.

Figure 6.1:5 F

The same injection sites as 6.1:5 E, after 6 days, showing spread in the vascular response, leading to loss of discrete inflammatory sites.

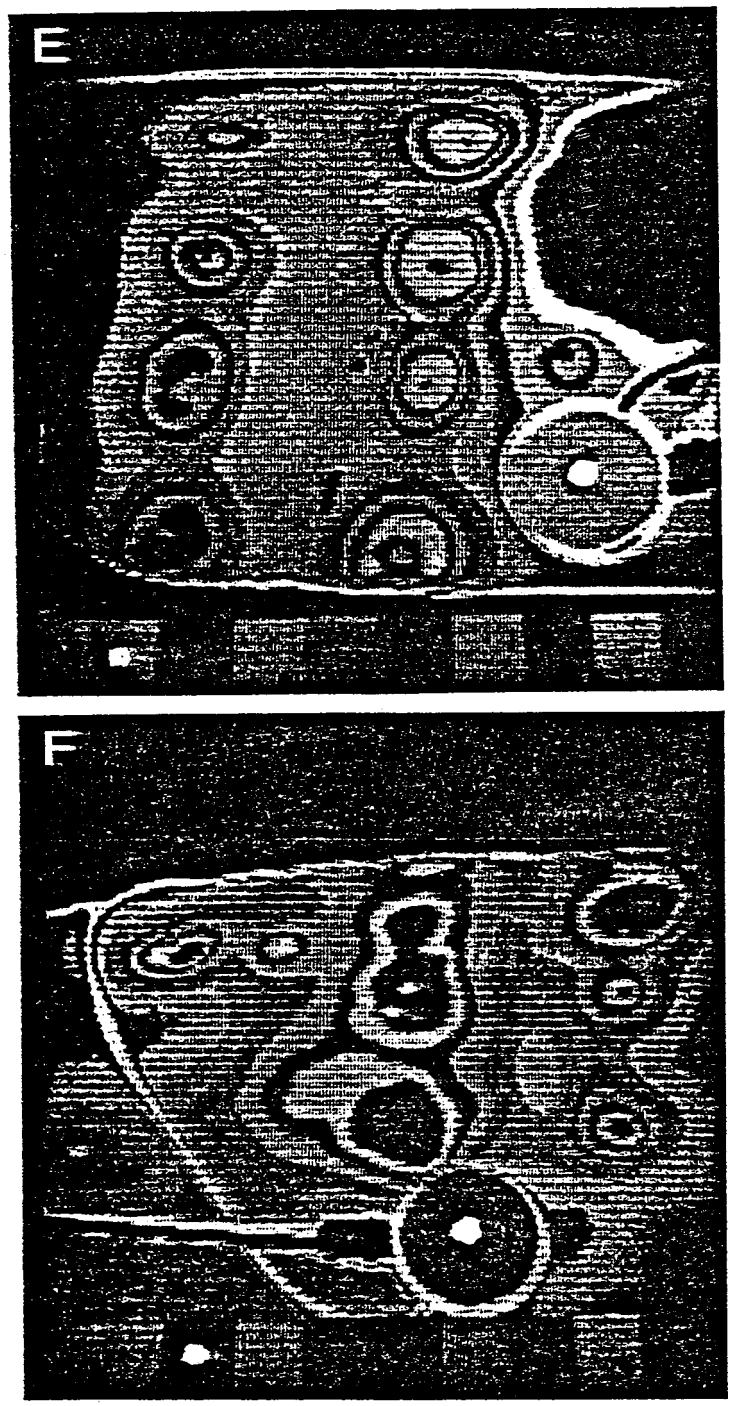


Figure 6.1:5 E and F

nomenclature. We recognize 3 strata (Figure 6.1:6). The first layer which lies on the basement membrane overlying the dermis has been called either the stratum basale or the stratum germinativum (Solkolov and Kalishnikova 1972, Ling 1974, Harrison and Thurley 1974). As both names appear to be freely interchangeable the term stratum germinativum will be used, as it is more common, and best describes function of the cells of this layer.

Stratum Germinativum

The epithelial cells and melanocytes of the stratum germinativum are attached to the basement membrane of the inter-digitating dermis by numerous hemidesmosomes. The epithelial cells are joined to adjacent epithelial cells with numerous desmosomes while melanocytes are sparingly joined to adjacent cells (Figure 6.1:6 and 6.1:7).

Epithelial cells of the stratum germinativum (Figure 6.1:7) have an oval-shaped nucleus enclosed in an undulent membrane which is often markedly indented by 1 μm diameter lipid droplets. Its fine structure is similar to other nuclei with a well-developed chromatin network, nuclear pores and one or more nucleoli. The cytoplasm contains many typical mitochondria concentrated particularly in the perinuclear area.

The protein synthesizing nature of these germinal cells is reflected by a small amount of rough endoplasmic reticulum and many free ribosomes. Elements of the Golgi apparatus are present in small number. A great bulk of the cell cytoplasm is taken up by a woven pattern of tonofibrils which crisscross the cytoplasm to enter the attachment plaques of desmosomes. The desmosomes are similar to those of human epidermis (Zelickson 1967). Harrison and Thurley (1974) describe a large intercellular space devoid of electron-dense material in Delphinus. However, the intercellar space in

our material (Tursiops truncatus) was quite small and contained fine granular electron-dense material.

Melanocytes (Figure 6.1:6 D) are larger and more circular than the adjacent germinatival cells. They have neither tonofibrils nor the numerous lipid droplets seen in the germinatival cells. The cytoplasm with many branching processes contains numerous mitochondria and melanin granules in various stages of synthesis.

Stratum Intermedium

Cells overlying the stratum germinativum make up what is variously called stratum intermedium, or the stratum spinosum (Sokolov and Kalishnikova 1972, Simpson and Gardner 1972, Ling 1974). Harrison and Thurley (1974) have subdivided this stratum into a lower stratum spinosum and an upper stratum intermedium. This separation of the intermediate layer of the epidermis is based on cell size and long axis orientation and not on either major ultrastructural or apparent functional differences between these layers. We use the term stratum intermedium to describe these layers collectively.

Cells of the stratum intermedium comprise the bulk of the epidermis. They show a gradual transition in form from spherical or ovoid near the stratum germinativum, becoming polyhedral at mid-level, and eventually elongate and flatten elliptically as they blend with the overlying stratum externum (Figure 6.1:6 B and 6.1:8).

The cytoplasm contains many more tonofibrils, and fewer mitochondria Golgi, and ribosomes than that of the germinativum (Figure 6.1:9 and 6.1:10 A). Numerous membrane-coating granules (Figure 6.1:10 B), mistaken by Sokolov and Kalishnikova (1972) to be mitochondria, are present at the periphery of the cells. There is also an increase in the number of

intercellular bridges and desmosomes (Figure 6.1:10 C and D).

The nuclei of these cells (Figure 6.1:9) are similar to those of the stratum germinativum with typical nucleoli still present. In some cells the nuclear membrane shows a greater indentation and there is a finer chromatin pattern.

Stratum Externum

The nature of the uppermost layer of cetacean epidermis is most controversial. Some authors (Palmer and Weddell 1964, Sperman 1972, Sokolov and Kalishnikova 1972 and Kleinenberg 1976) believe that cornification (keratinization) of the uppermost layer takes place, though they present no direct evidence. Others (Simpson and Gardner 1972, Harrison and Thurley 1974) suggest that the upper layer of cetacean skin is not cornified. We support the latter view, and use the term stratum externum (Harrison and Thurley 1974) rather than stratum corneum.

The stratum externum is the thin outermost portion of epidermis consisting of 30 to 50 layers of extremely flattened cells (Figure 6.1:6 and 6.1:11), most of which have small condensed nuclei (Figure 6.1:12 B). There is marked peripheral clumping of the chromatin and the nuclear membrane appears to be intact.

The cytoplasm is composed primarily of densely-packed tonofibrils. Harrison and Thurley (1974) indicated the presence of mitochondria, melanin granules, and membrane-coating granules in the cytoplasm of these cells. However, we found these to be very rare in our samples.

Figure 6.1:6 A

Full thickness of the epidermis of the dolphin, Tursiops truncatus. The three epithelial divisions, the stratum externum (SE), stratum intermedium (SI), and the single row of cells comprising the stratum germinativum (SG) are illustrated. The dermal papillae (DP) of the dermis (D) interdigitate with the rete pegs (RP) of the epidermis. H and E x 180.

Figure 6.1:6 B

Photomicrograph of the stratum externum (SE). The faint horizontal striations represent the intercellular bridges adjoining the layers of cells. The large, dark, circular spots are lipid droplets (arrows) and the faint elliptical structures are nuclei (arrow head). The nuclei (N) of the stratum intermedium (SI) are more apparent. Methylene blue, 1 μ. x 1400

Figure 6.1:6 C

Photomicrograph of the stratum intermedium showing the characteristic polygonal shape of the cells of the stratum. The nuclei are large and round and often contain a prominent nucleolus (arrow). The intercellular bridges form a dense palisade around each cell (arrow head). Methylene blue 1 μ. x 1600

Figure 6.1:6 D

The stratum germinativum (SG) is a single layer of cells attached to the basement membrane (arrow) overlying the well-vascularized (V) dermis (D). The stratum germinativum contains both epithelial cells (E) and large, round melanocytes (M). Methylene blue, 1 μ. x 1600

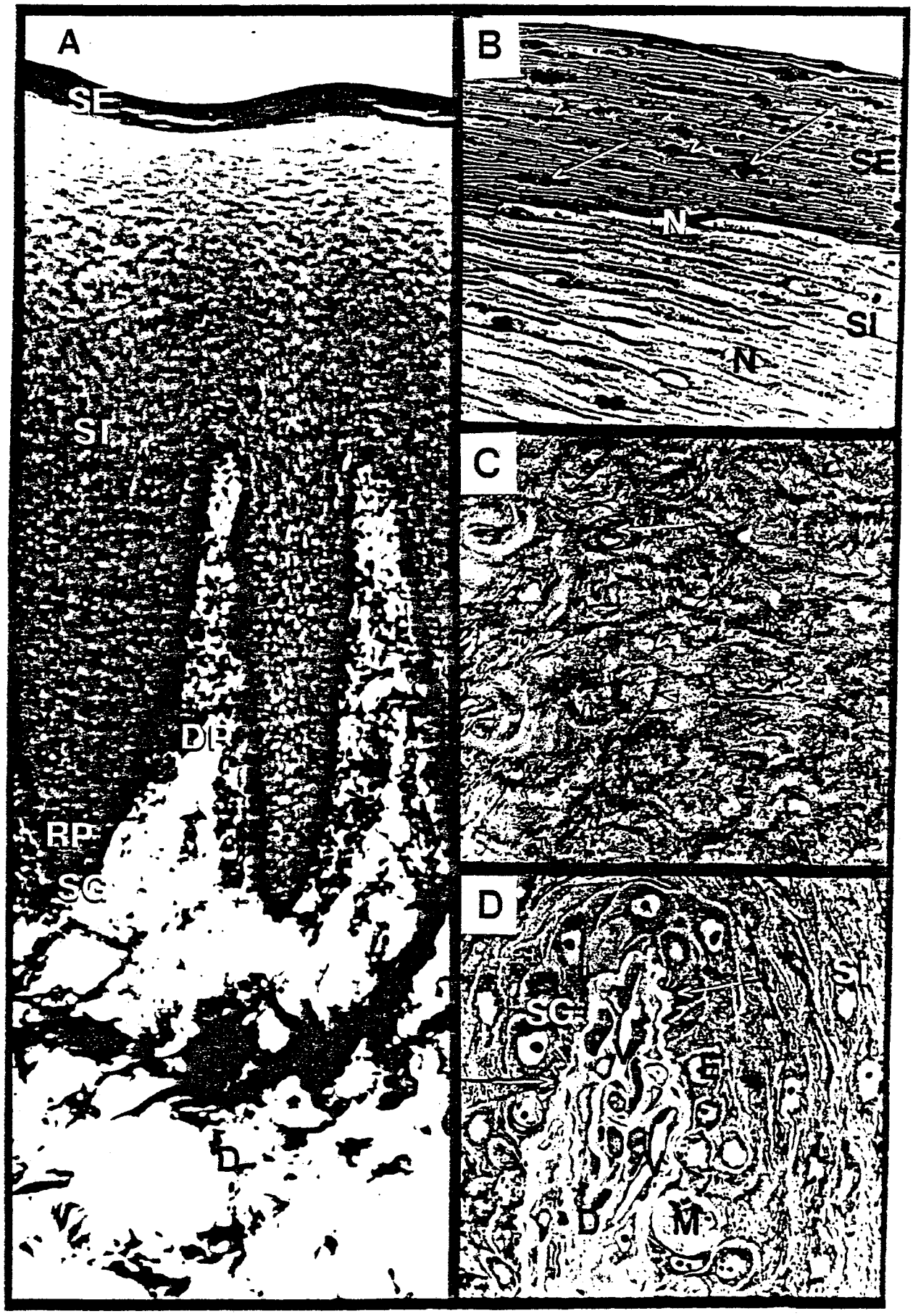

A
SE
ST
DP
RP
SG
D
B
SE
N
SI
N
C
D
SG
ST
D
M

Figure 6.1:7

An epithelial cell of the stratum germinativum, attached to adjacent epithelial cells with well-developed intercellular bridges (IB) and resting on the basement membrane (BM) overlying the collagen-rich dermis (D). The cytoplasm contains many tonofibrils (TF), free ribosomes (arrow), some mitochondria (M), and several perinuclear and apical lipid droplets (L). The nucleus (N) has a prominent nucleolus (Nu). x 12,000

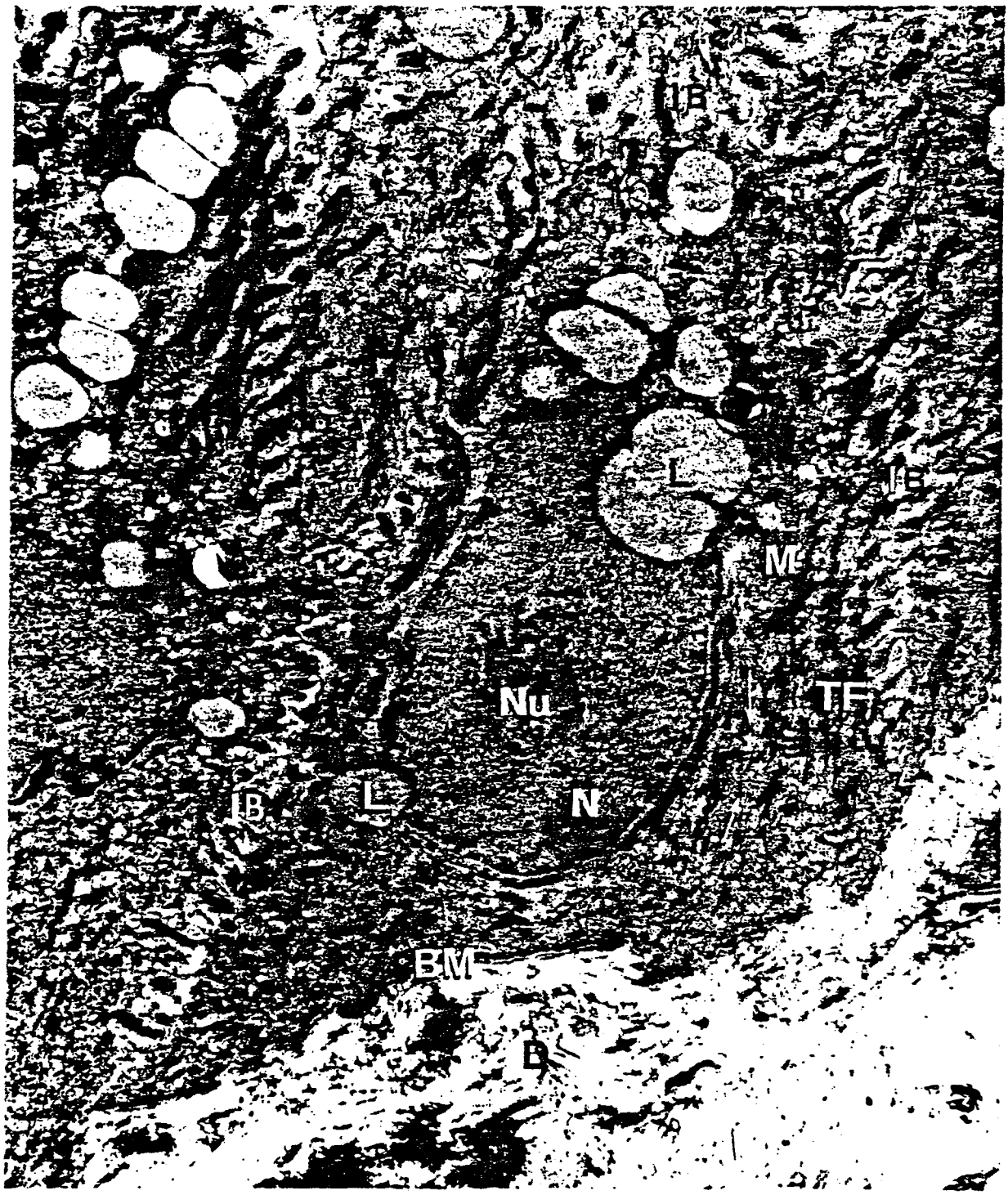


Figure 6.1:7

Figure 6.1:8 A

Upper reaches of the stratum intermedium depicting the elongation of the cells (arrows show distal and proximal cell boundaries) and its nucleus. Fewer and apparently larger lipid droplets are present. There is also a paucity of organelles within the cytoplasm. x 5600

Figure 6.1:8 B

Mid layer of stratum germinativum with typical polygonal-shaped cells, with moderate numbers of cytoplasmic organelles. x 5600

Figure 6.1:8 C

Proximal region of the stratum spinosum illustrating a more spherically-shaped cell. The nucleus and cytoplasm have similar characteristics to those in the mid layer (Figure 6.1:8 B). x 5300

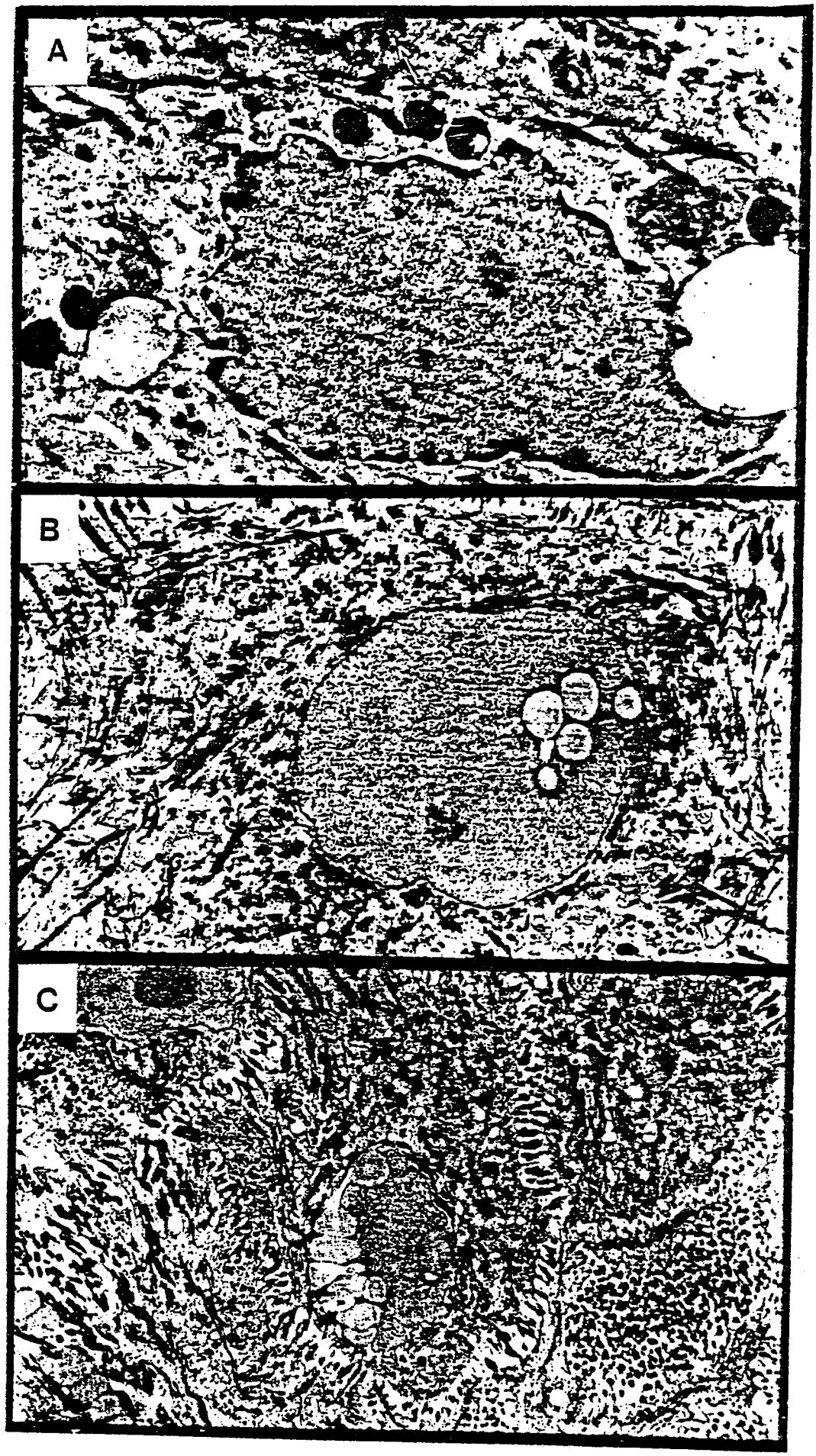

A
B
C

Figure 6.1:9

Cells of the mid layers stratum intermedium have round to oval-shaped nuclei (N) with a fine chromatin pattern and prominent nucleoli (Nu). Lipid droplets (L) are often present in infolded area of the nucleus. The cytoplasm contains some mitochondria (M), membrane-coating granules and an extensive array of crisscrossing tonofibrils (TF). Closely stacked, well-developed intercellular bridges (IB) outline the periphery of the cell. x 7500

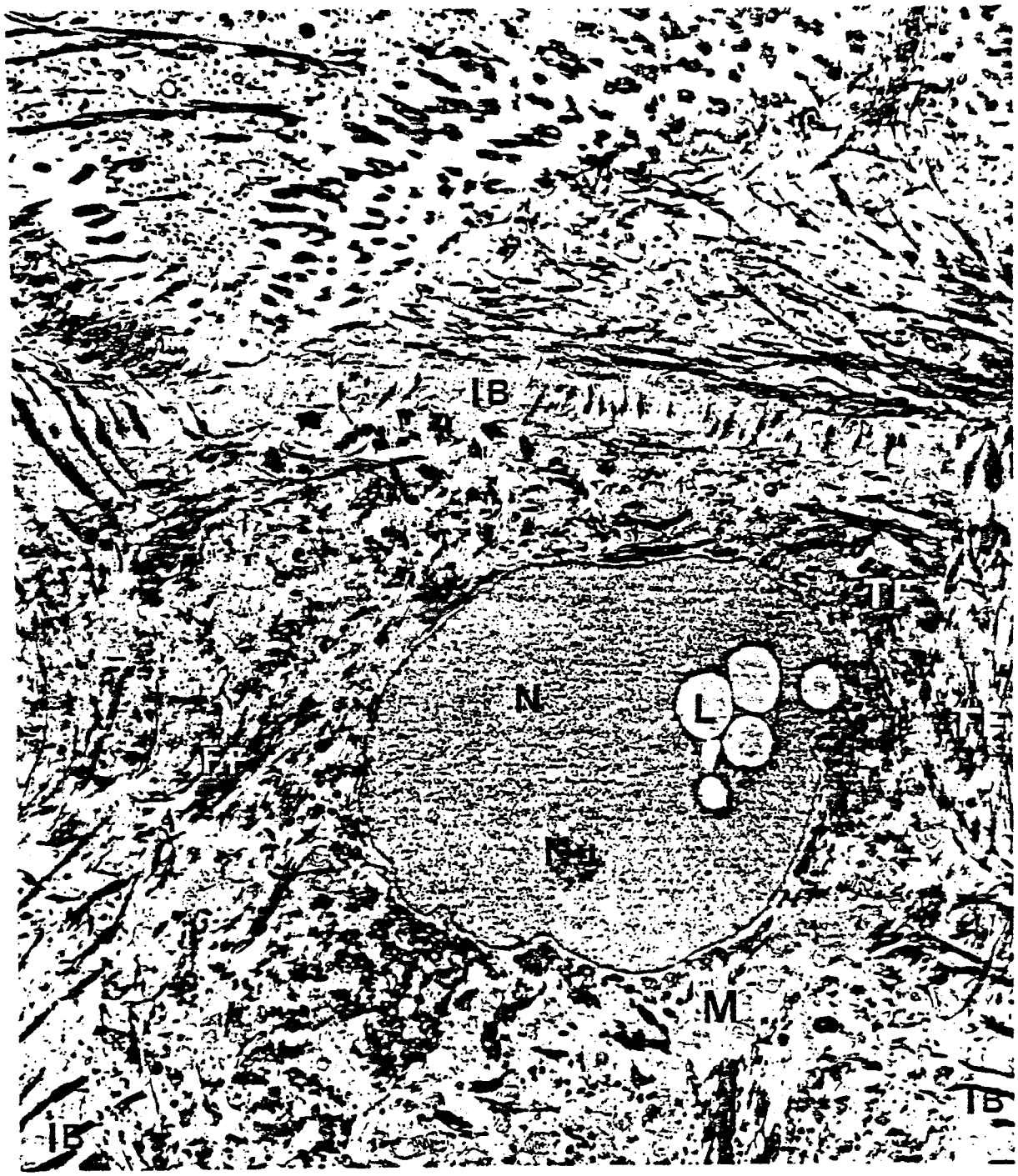


Figure 6.1:9

Figure 6.1:10 A

Some Golgi complexes (G) are present adjacent to the nucleus in cells of the stratum intermedium. These are accompanied by free ribosomes (arrow) and mitochondria (M). x 32,500

Figure 6.1:10 B

Detail of the lamellar structure of the membrane-coating granules found in the cells of the stratum intermedium. x 140,000

Figure 6.1:10 C

Fine structural detail of desmosomes (De) and intercellular bridges (IB) of the stratum intermedium cut parallel to the cell surface. x 125,000

Figure 6.1:10 D

Desmosomes in the stratum intermedium, cut in a plane perpendicular to the cell surface showing the attachment of the structural units of the tonofibrils (TF), the tonofilaments, to the attachment plaque. x 35,000

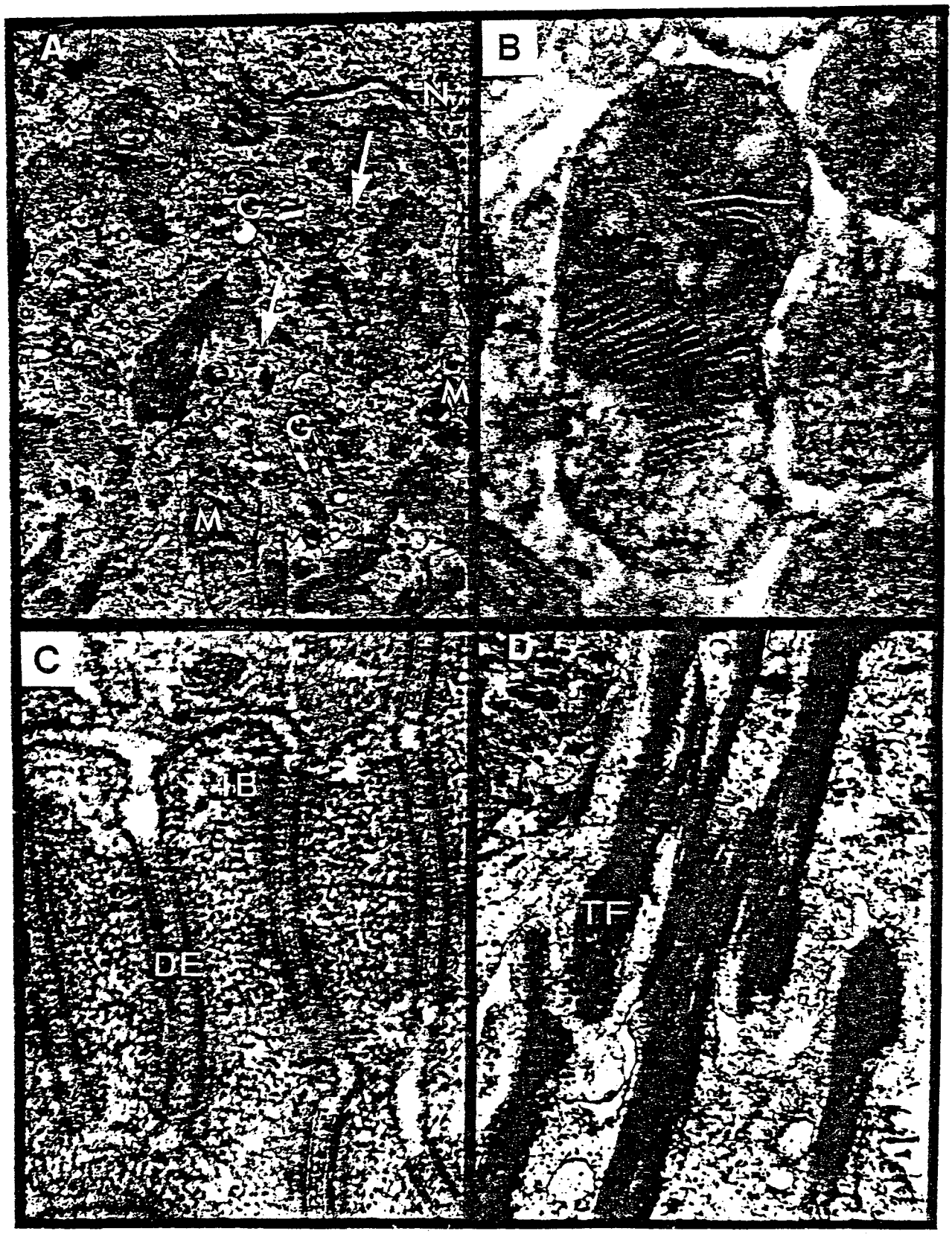

A
N
C
M
G
M
B
C
B
DE
D
TF

Figure 6.1:11

The stratum externum (SE). Multiple layers of flattened cells are separated by light striations. Cells adjacent to the stratum intermedium (SI) contain large nuclei (N), while the nuclei of cells in the upper layers are less prominent (arrows). The large, dark globules are lipid droplets, and the small particles are melanin granules. x 400

Figure 6.1:11

Figure 6.1:12 A

The uppermost layers of exfoliating stratum externum cells have a condensed filamentous cytoplasm containing very few organelles, and some lipid droplets (L). The intercellular bridges disarticulate, and become villous projections (arrow) along the cell margins. x 12,000

Figure 6.1:12 B

Cells in the mid region of the stratum externum have nuclei (N) which contain coarsely clumped chromatin. A few large lipid droplets (L) remain in the cytoplasm and there is a paucity of normal cell organelles. The intercellular bridges are reduced in size and are oriented more parallel with the cell surface. x 15,000

Figure 6.1:12 C

Cells of the deeper region of the stratum externum contain more normal appearing nuclei. The cytoplasm and intercellular bridges (IB) have a transitional appearance between those of the stratum intermedium illustrated in Figure 6.1:9 and those of the upper portions of the stratum externum illustrated in Figures 6.1:12 A and B. x 12,500

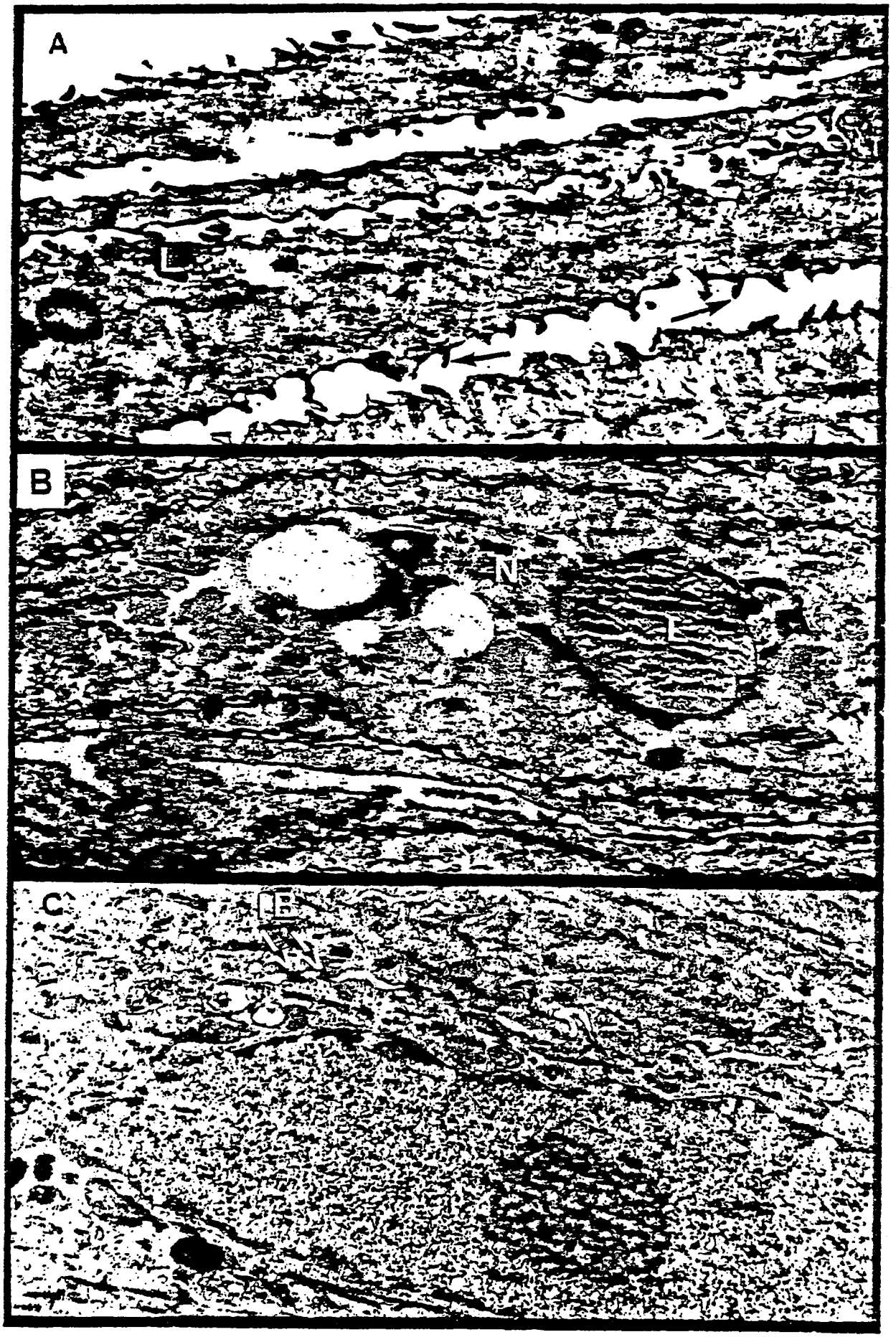


Figure 6.1:12 A-C

The desmosomes are more densely arranged than in the stratum intermedium. The components of the desmosomes are not distinct, nor is the attachment plaque. The cells on the surface exfoliate as the intercellular bridges disarticulate (Figure 6.1:12 A).

In summary, cetacean epidermis is a histologically simplified version of the epidermis of other mammals, with certain unique features. There is no cornified (keratinized) upper layer. The stratum spinosum is many cell layers thick. There are neither hair follicles nor sweat glands in the mature cetacean. The specialized Langerhans and Merkel cells are not observed. Numerous dermal papillae, arranged in craniocaudal ridges, project two-thirds of the way up into the thick epidermis. In our description, we use the terms stratum germinativum, stratum intermedium, and stratum externum to describe three subdivisions of cetacean epidermis.

Histological Changes in Cetacean Skin Following Exposure to Petroleum Hydrocarbons

The cellular response to the application of crude oil and lead-free gasoline was judged by examining up to 12 biopsy samples taken at hourly and daily intervals for up to 7 days (Table 6.1:4). Lead-free gasoline caused minimal to moderate changes. In the stratum externum, these included disruption of intercellular bridges leading to premature separation of cells (Figure 6.1:13 and 6.1:14), the presence of floccular densities (Figure 6.1:14) within the cytoplasm, vacuolization, aggregation of tonofilaments, and clumping of nuclear chromatin (Figure 6.1:15).

The cytoplasm of the cells of the stratum intermedium showed a variable pattern of rarefaction, vacuolization, and floccular densities

Table 6. 1:4 Schedule of skin biopsy excisions from *Tursiops truncatus* and *Grampus griseus* following exposure to petroleum hydrocarbons

Petroleum Hydrocarbon	Exposure time (min.)	Biopsy sampling time after petroleum hydrocarbon exposure												
		0	30 min.	1 hr.	2 hrs.	4 hrs.	8 hrs.	1 day	2 days	3 days	4 days	5 days	6 days	7 days
W-T crude oil[1]	25	BIM-A[4]												
W-T crude oil	25	BIM-B												
W-T crude oil	30	BIM-C	BIM-C	BIM-C	BIM-C	BIM-C	BIM-C	BIM-C	BIM-C	BIM-C	BIM-C	BIM-C	BIM-C	BIM-C
I-P crude oil[2]	45	BIM-A												
L-F gasoline[3]	15	BIM-A												
L-F gasoline	15	BIM-B												
L-F gasoline	15	BIM-C[5]		BIM-C	BIM-C	BIM-C	BIM-C	BIM-C						
L-F gasoline	30	BIM-A									BIM-A			BIM-A
L-F gasoline	30	BIM-C									BIM-C			BIM-C
L-F gasoline	45	BIM-A		BIM-A	BIM-A	BIM-A	BIM-A	BIM-A						
L-F gasoline	30	BIM-C									BIM-C			BIM-C
L-F gasoline	45	BIM-A		BIM-A	BIM-A	BIM-A	BIM-A	BIM-A						
L-F gasoline	45	BIM-B												BIM-B
L-F gasoline	45	*Grampus*							*Grampus*		*Grampus*			
L-F gasoline	60	BIM-A												BIM-A
L-F gasoline	60	BIM-C												BIM-C
L-F gasoline	75	BIM-C								BIM-C				

[1] West-Texas crude oil
[2] Inter-provincial crude oil
[3] Lead-free gasoline
[4] BIM-A, B, C refer to BIM numbers assigned to IDR *Tursiops*
[5] There were 2 biopsies removed at this exposure duration from this *Tursiops*

Figure 6.1:13 A

Photomicrograph of the stratum externum and upper layers of the stratum intermedium. There is marked separation of some of the cells of the stratum externum due to widespread intercellular vacuolization. Sample taken from dolphin BLM-C immediately after 30 min. exposure to lead-free gasoline. Methylene blue, 1 µ. x 400

Figure 6.1:13 B

Electronmicrograph of a portion of stratum externum similar to that illustrated in Figure 6.1:15 A. Intercellular swelling has led to the disruption of numerous intercellular bridges while others have remained intact (arrow). Sample taken from dolphin BLM-C immediately after 30 min. exposure to lead-free gasoline. x 5,100

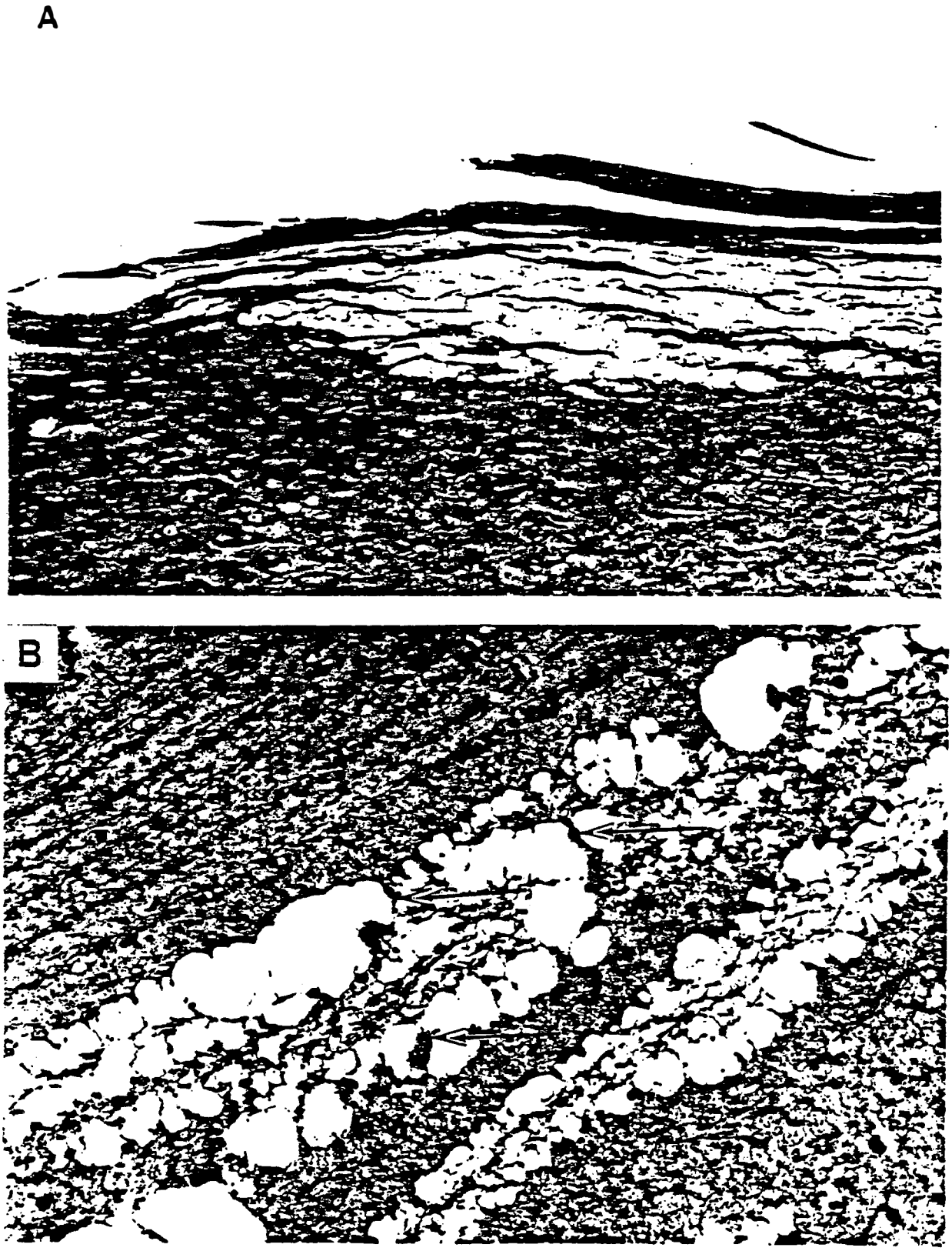


Figure 6.1:13 A and B

Figure 6.1:14 A

Junction between the stratum externum and the stratum intermedium. Note the marked increase in intercellular space and the presence of numerous floccular densities (arrow). Sample taken from dolphin BLM-B immediately after 45 min. exposure to lead-free gasoline. x 12,500

Figure 6.1:14 B

Control sample of a similar region of skin from dolphin BLM-C. There is a minimal amount of intercellular space (arrow) and an abundance of fine tonofilaments. x 12,500

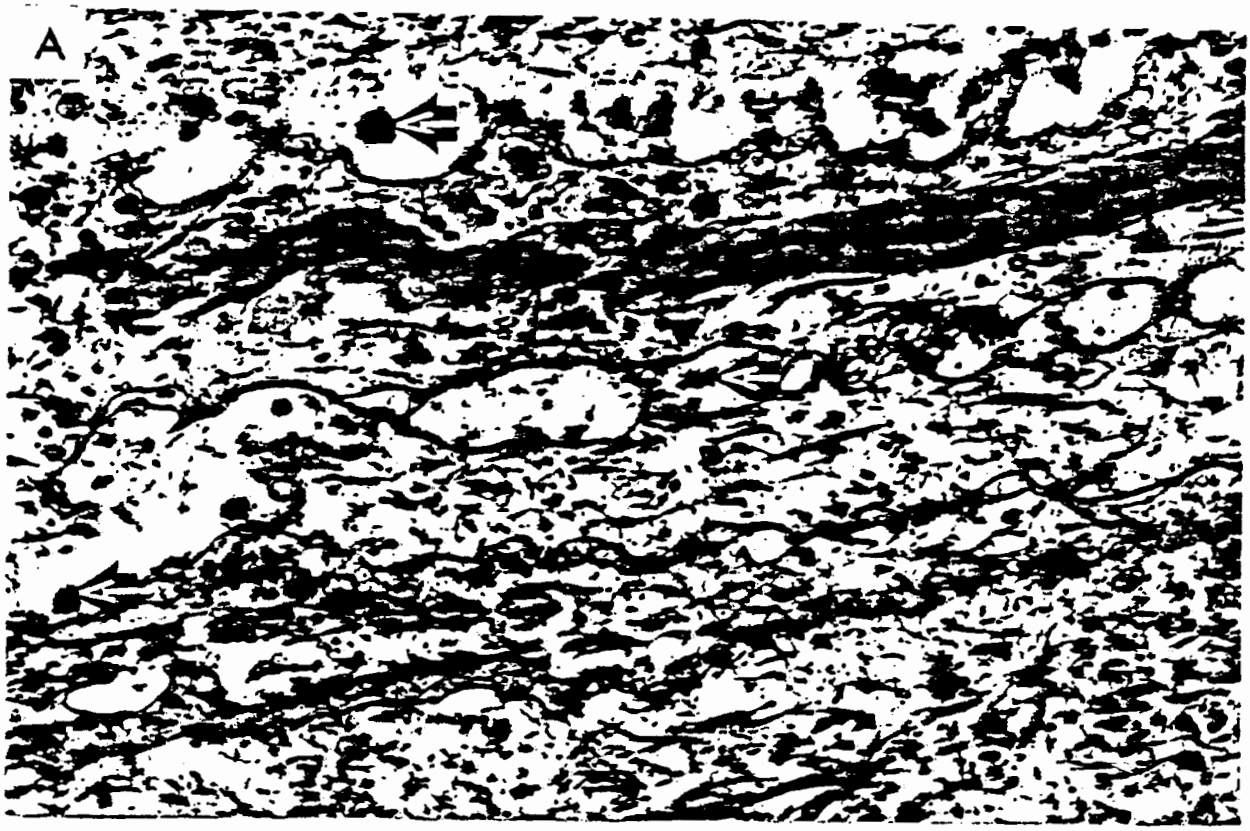


Figure 6.1:14 A and B

Figure 6.1:15 A

Deep layer of the stratum externum illustrating aggregation of tonofilaments, increased intercellular space (X) and clumping of nuclear chromatin (NC). Sample taken from dolphin BLM-A immediately after 15 min. exposure to lead-free gasoline. x 12,500

Figure 6.1:15 B

Control sample from dolphin BLM-A, illustrating normal ultrastructure of the deep layer of the stratum externum. x 12,500

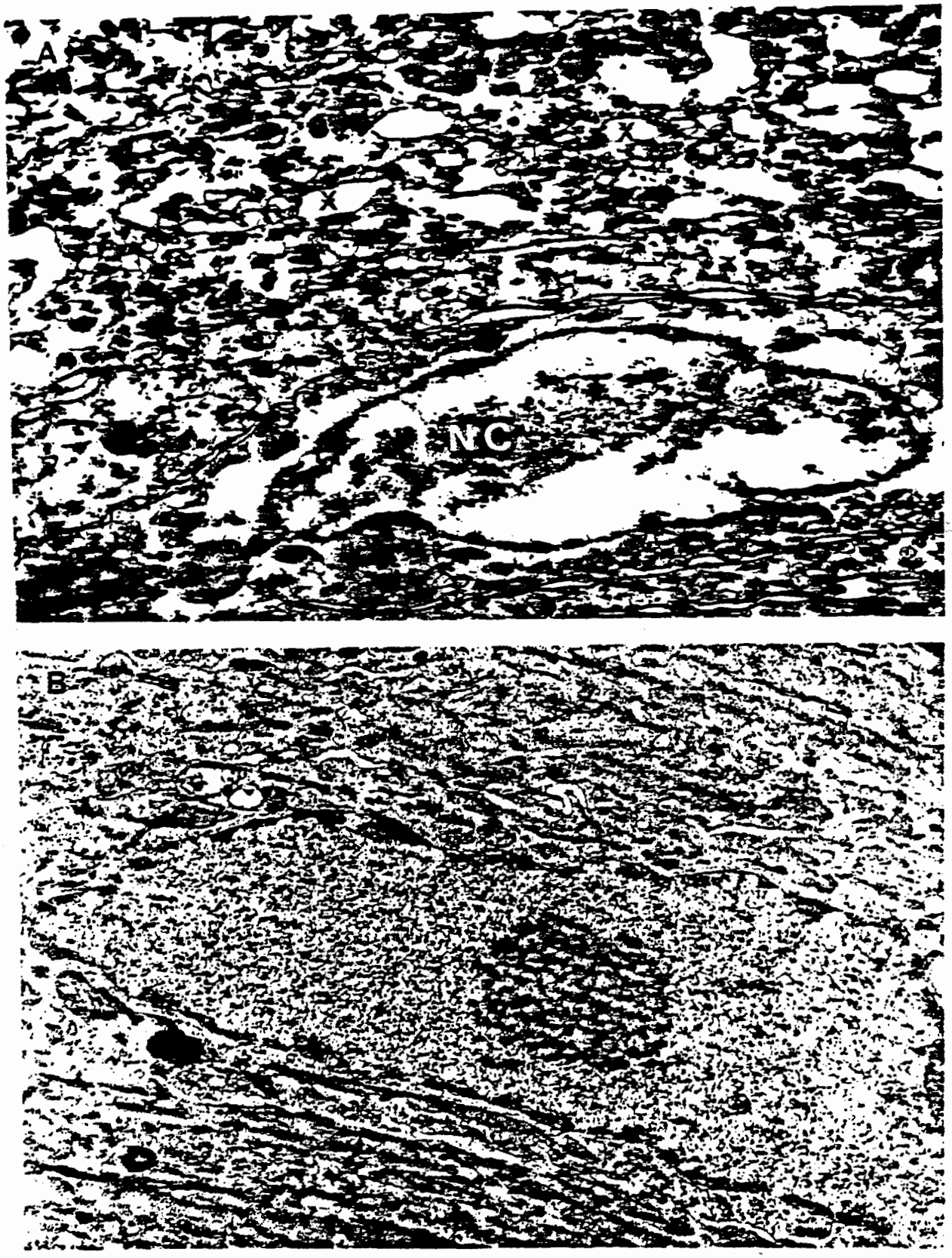


Figure 6.1:15 A and B

(Figure 6.1:16 A and B). Nuclei of affected cells showed chromatin aggregation, rarefaction, (Figure 6.1:16 A) and in some cases, extensive vacuolization and fragmentation of the nuclear membrane (Figure 6.1:16 B). In select samples, intercellular bridges were disrupted within the stratum intermedium (Figure 6.1:17 B). In the 75 minute exposure trials there was increased density of the ground substance comprising the intercellular bridges (Figure 6.1:18).

Notable changes in the stratum germinativum were present only in the skin exposed to gasoline for 75 minutes. There was marked rarefaction of nuclei (Figure 6.1:19) and perinuclear cytoplasm, and some mild aggregation of tonofilaments.

In dermis, pathologic changes were observed only after the skin had been exposed to lead-free gasoline for 75 minutes. The fibrocytes showed extensive cytoplasmic rarefaction. The collagen fibers appeared to be swollen and more electron-dense.

Samples collected 3, 4, or 7 days after exposure to lead-free gasoline showed ultrastructural characteristics consistent with cell recovery following sublethal intoxication. These changes included intra- and intercellular residual bodies resulting from the breakdown of protein. Figures 6.1:20 A, B and C illustrate these structures in the stratum intermedium, stratum germinativum, and dermis respectively.

The changes in dolphin skin exposed to gasoline indicate sublethal cytotoxicity (Trump and Arstilla 1975). The presence of floccular electron-dense bodies within the cytoplasm, perinuclear cisternae (vacuoles), clumped or aggregated tonofilaments, cytoplasmic and nuclear rarefaction, enlarged intercellular space and disruption of desmosomes in

Figure 6.1:16 A

Cells of the stratum intermedium showed nuclear rarefaction and numerous small aggregations in the cytoplasm (arrow). Sample taken from dolphin BLM-B immediately after 45 min. exposure to lead-free gasoline. x 6,100

Figure 6.1:16 B

Some cells in the stratum intermedium showed marked vacuolization of the nucleus, and fragmentation of the nuclear membrane, in addition to cytoplasmic changes, as described above. Some large vacuoles (V) are also present in the intercellular space. Sample taken from dolphin BLM-A immediately after 15 min. exposure to lead-free gasoline. x 5,100

Figure 6.1:16 C

Control sample from dolphin BLM-A illustrating normal cell in the stratum intermedium. x 5,100

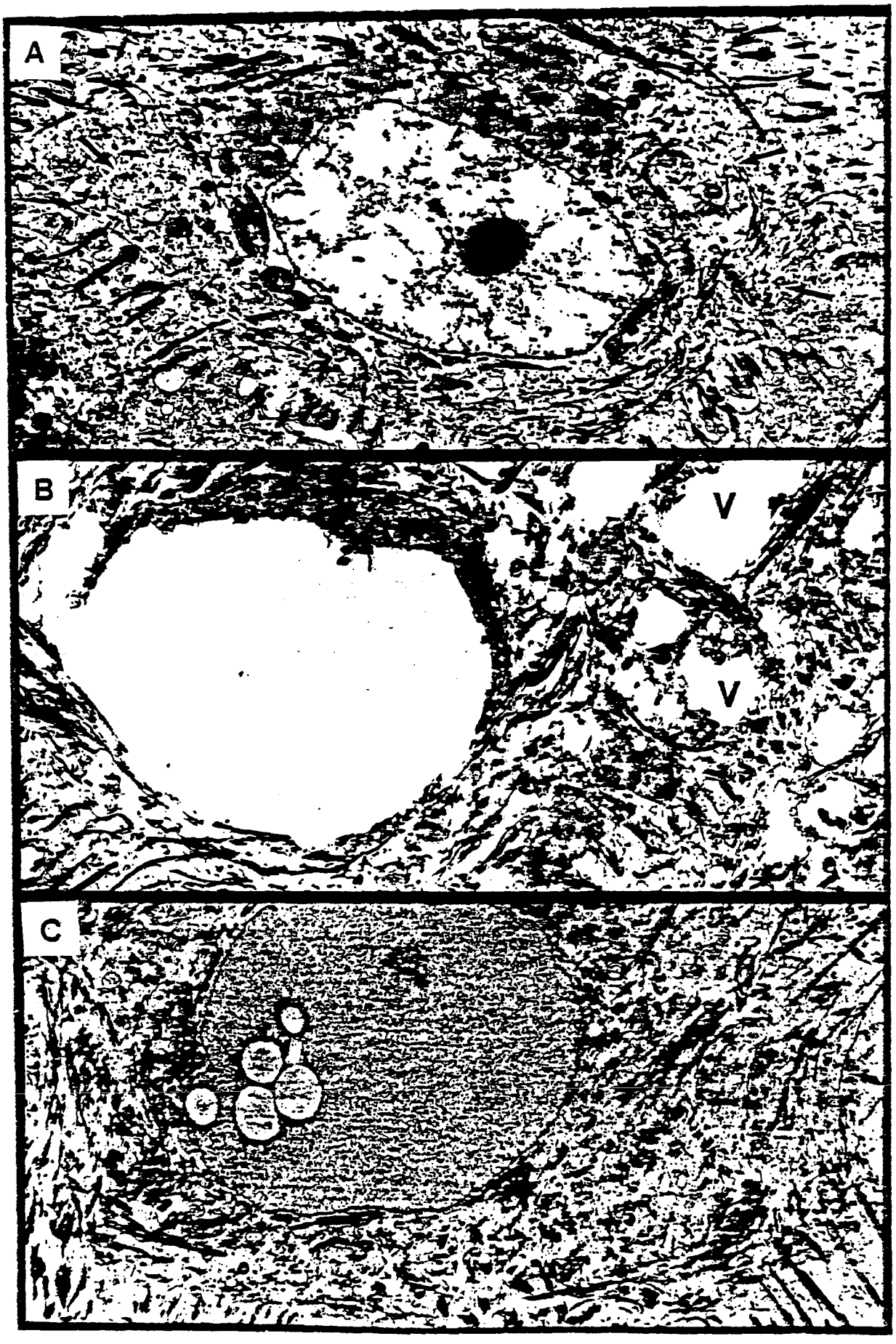

A
B
V
V
C

Figure 6.1:17 A

Thin section of stratum intermedium illustrating increased intercellular space (arrow). Sample taken from dolphin BLM-C 3 days after 75 min. exposure to lead-free gasoline. Methylene blue 1 µ. x 1,000

Figure 6.1:17 B

Electronmicrograph of a section similar to that shown in Figure 6.1:17 A, demonstrating the fine structure of the intercellular bridge breakdown. Desmosomes are disrupted resulting in an increase in intercellular space. Sample taken from dolphin BLM-C, 3 days after 75 min. exposure to lead-free gasoline. x 12,500

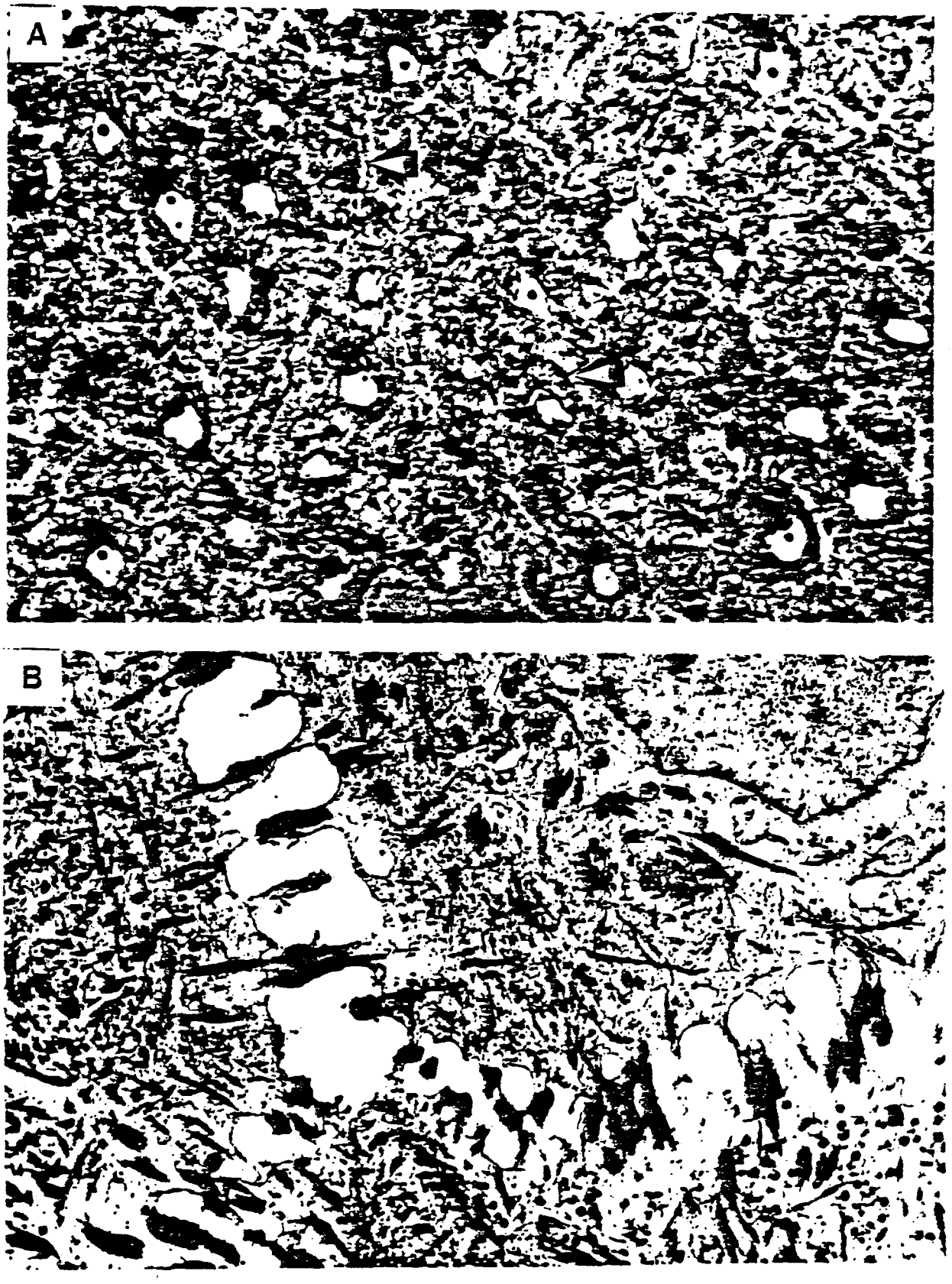


Figure 6.1.17 A and B

Figure 6.1:18 A

Photomicrograph of deep stratum intermedium (SI), stratum germinativum (SG), and dermal papilla (DP). Note the dark thin intercellular matrix (arrow). Many of the cells of the stratum germinativum show marked cytoplasmic rarefaction (double arrow). Sample taken from BLM-C 3 days after 75 min. exposure to lead-free gasoline. Methylene blue, 1 µ. x 1,000

Figure 6.1:18 B

Electronmicrograph of a similar area of stratum intermedium as that illustrated in Figure 6.1:18 A. Ultrastructural detail of the dense intercellular matrix and the perinuclear vacuolization are illustrated. Sample taken from dolphin BLM-C 3 days after 75 min. exposure to lead-free gasoline. x 6,600

Figure 6.1:18 A and B

Figure 6.1:19 A

Cytoplasmic rarefaction in cells of the stratum germinativum is most marked in the perinuclear region (CR). Clumping of tonofilaments is also present (arrow). Sample taken from dolphin BLM-C immediately after 75 min. exposure to lead-free gasoline. x 5,600

Figure 6.1:19 B

Stratum germinativum (SG) and adjacent dermis (D), which contains much collagen, taken from a non-exposed area on dolphin BLM-A. x 6,300

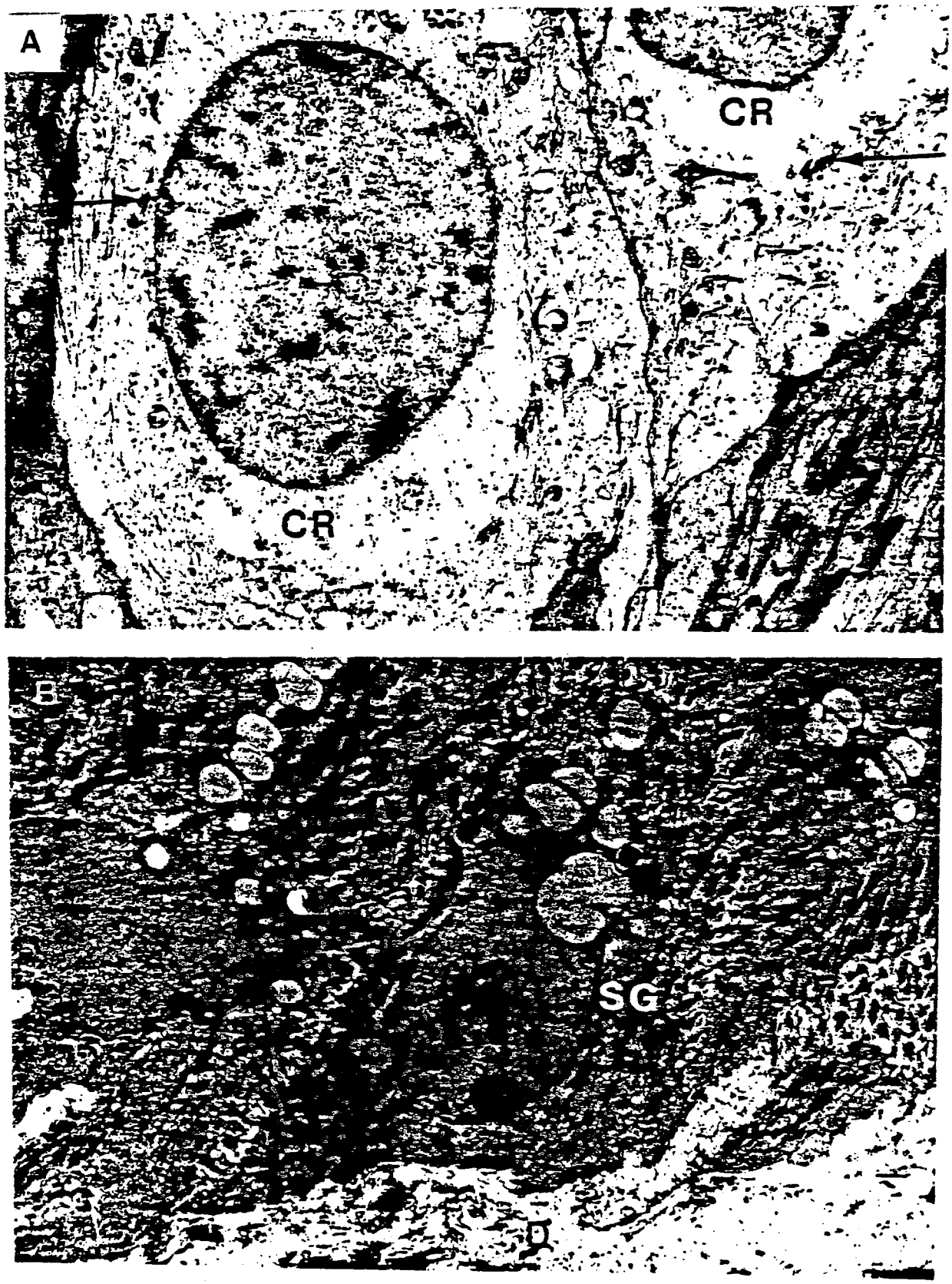


Figure 6.1:19 A and B

Figure 6.1:20 A

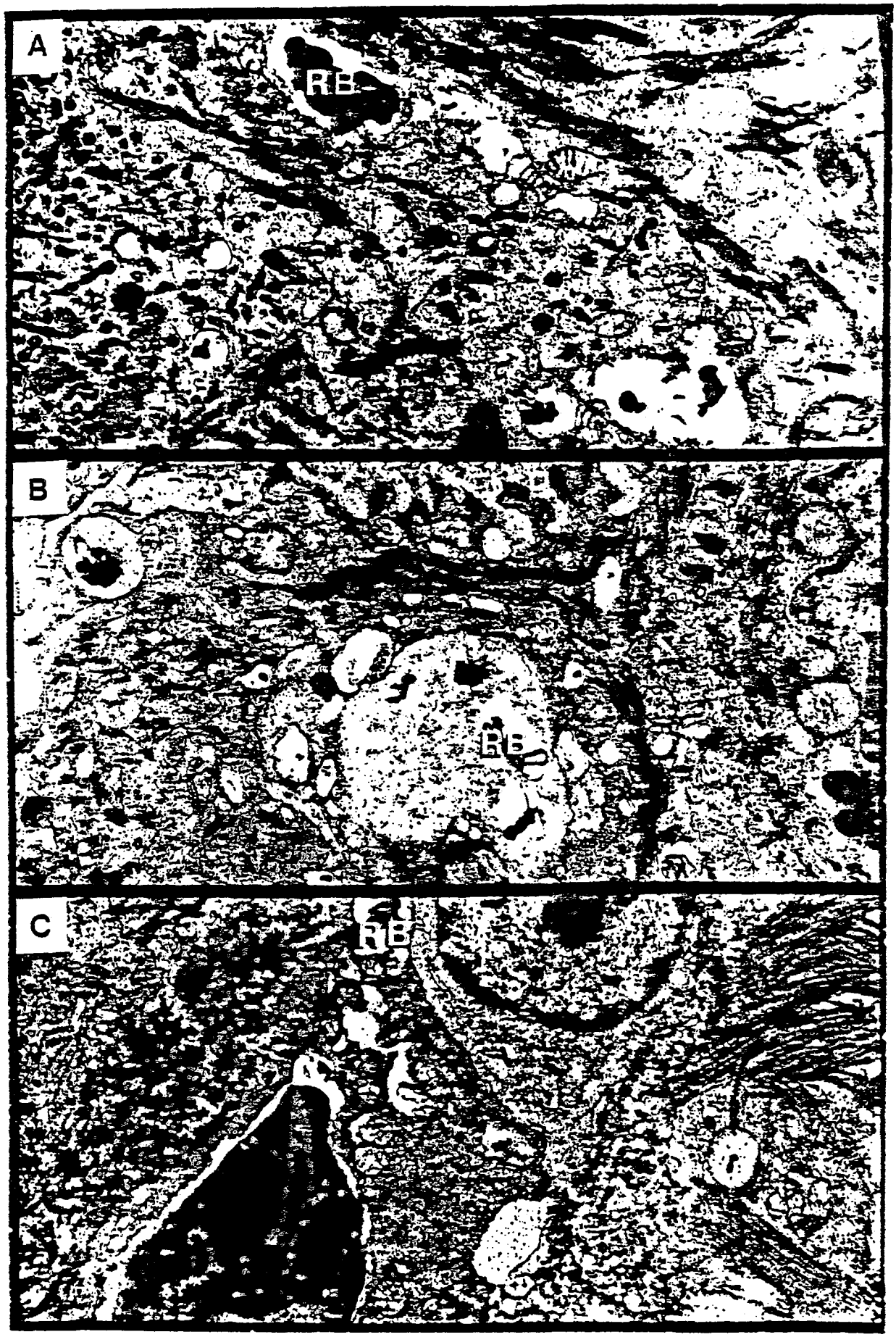
Stratum intermedium contain numerous membranous laminated residual bodies in cytoplasmic vacuoles (RB). Sample taken from dolphin BLM-C 3 days after 75 min. exposure to lead-free gasoline. x 9,000

Figure 6.1:20 B

Stratum germinativum cells contain numerous residual bodies in cytoplasmic vacuoles (RB). Sample taken from dolphin BLM-C 3 days after 75 min. exposure to lead-free gasoline. x 9,000

Figure 6.1:20 C

Residual bodies (RB) are present in cytoplasmic vacuoles within fibrocytes in the dermis. Sample taken from dolphin BLM-B 7 days after 45 min. exposure to lead-free gasoline. x 14,000


A
RB
B
RB
C
RB

affected cells are consistent with previously published observations on acetone and kerosene exposure in humans (Lupulescu et al. 1973).

Recovery of epidermal cells after acute exposure to gasoline appears to be rapid. The presence of residual bodies within intracellular vacuoles suggests that some damage to cellular proteins and lipids has resulted in the formation of autophagosomes (Threadgold 1976). The rapid recovery of exposed skin both grossly and histologically, confirms the sublethal nature of the injury and is indicative of full restoration.

Effects on Wound Healing of Prior Exposure to Petroleum Substances

The results of the study are shown in Tables 6.1:5, 6 and 7. Wedge biopsies were always associated with some degree of bleeding. This was controlled with hemostatic powder[10] applied directly into the wound. The first observations made 2 days (Grampus) and 3 days (Tursiops) after the biopsies were excised, were similar in appearance irrespective of the petroleum product used. The edges were smooth and they appeared dark gray to black in color. A halo of progressively paler gray surrounded the wounds. The base of each wound consisted of a firm, cream-colored core. There was a gradual reduction in size of the wound opening and the surrounding halo over the course of healing. Generally, the wounds were closed by 7 to 10 days, though a faint halo persisted around the fine black linear scar for the 14 day period of observation.

There was no observable difference in the healing process between nonexposed skin, and skin that had been exposed to the 3 petroleum products.

Table 6.1;5 Healing progress of biopsy site after exposing Tursiops skin to crude oil for 25 and 45 minutes

Crude Oil	Exposure Time (min.)	Animal Number	Time After Exposure (days)					
			3	7	10	14	17	21
W-T crude oil[1]	25	BLM-A	-firm, custard colored plug in wound. Irregularly circular pale gray halo -slightly raised	- reduction in size of yellow plug - raised dark gray wound edges	-dark gray edges beginning to close -no yellow plug	-wound closed -dark gray area follows contours of scar	--	--
		BLM-B	-pale gray halo follows contours of wound -smooth, dark gray-black wound edges	-small, firm custard-yellow plug in wound -dark gray edges beginning to close	-no yellow plug -dark gray wound edges closing	-wound closed -dark gray area follows contours of scar	--	--
I-P crude oil[2]	45	BLM-A	-pale gray, irregularly circular area around wound -slightly raised dark gray wound edges	-pale gray halo following contours of wound -dark gray, slightly raised edges are starting to close	-dark gray edges -wound almost closed	-wound has opened -wound edges are dark gray, wrinkled and raised -bead-sized yellow plug	-dark gray wound edges fusing -no yellow plug	-wound has closed -slightly darker gray area follows contours of scar

[1] W-T crude oil: (50-307) West Texas Crude

[2] I-P crude oil: Inter-provincial Crude

Table 6.1:6 Healing progress of biopsy site after exposing Tursiops skin to lead-free gasoline for 15, 30, 45, 60 and 75 minutes

Exposure Time (min.)	Animal Number	Time After Exposure (days)			
		3	7	10	14
15	BLM-A	-smooth, clean wound -edges beginning to close -no discoloration	-wound closed -dark gray linear scar	—	—
	BLM-B	-pale gray halo following contours of wound -dark gray wound edges are slightly raised	-dark gray edges almost completely closed	-wound has closed -dark gray linear scar	—
	BLM-C	-pale gray halo following contours of wound -smooth, clean dark gray wound edges	-wound closed -dark gray linear scar	—	—
30	BLM-A I	-pale gray halo following contours of wound -slightly raised dark gray wound edges	-slightly raised -dark gray wound edges beginning to close	-dark gray wound edges closing	-wound almost completely closed
	BLM-A II	-firm, custard yellow plug in wound -slightly raised -pale gray halo following contours of wounds	-yellow plug absent -dark gray edges are wrinkled, beginning to close	-dark gray edges are smooth -almost complete closure of wound	—
	BLM-A III	-pale gray halo follows contours of wound -smooth, clean, dark gray wound edges	-dark gray edges of wound beginning to close	—	—
	BLM-C I	-no discoloration; smooth, clean, dark gray wound edges beginning to close	-area slightly raised -firm, custard yellow plug in wound	-yellow plug disappeared -dark gray wound edges closing; wrinkled edges	-wound almost completely closed
	BLM-C II	-firm, custard-yellow plug in wound -slightly raised -dark gray wound edges are wrinkled	-yellow plug disappeared -slightly raised -dark gray wound edges are beginning to close	-wound almost completely closed	—
	BLM-C III	-smooth, clean dark gray wound edges beginning to close	-wound almost completely closed; dark gray edges contacting	—	—

Table 6.1:6 continued

Exposure Time (min.)	Animal Number	Time After Exposure (days)	
		3	7
45	BLM-B	-smooth, clean, dark gray wound edges beginning to close	-wound closed -dark gray linear scar
60	BLM-C	-smooth, clean, dark gray wound edges beginning to close	-wound closed -dark gray linear scar
	BLM-A	-firm, custard-yellow plug in wound -wrinkled, dark gray edges of wound -beginning to close	-wound nearly closed
75	BLM-B	-*irregularly-shaped dark gray halo -incision barely noticeable	—
	BLM-C	-firm, custard-yellow plug in wound -smooth, dark gray wound edges beginning to close	—

* The incisions from the wedge biopsy were made but the section of skin was not removed.

Table 6.1:7 Healing progress of biopsy sites after exposing Grampus skin to lead-free gasoline for 45 minutes

Biopsy Site	Number of Days Following Biopsy Excision			
	Wedge		Dermotomy	
	2	4	2	4
control	--	--	-shallow crater -continuous in consistency and color with surrounding tissue	-slight depression in skin -no change
gas exposed	-clean, smooth, dark gray to black edges, beginning to close -elevated temperature of immediate area	-smooth, clean, dark gray to black edges -small, raised granular, pink area in center of wound -elevated temperature of immediate area	-slightly darker gray irregular rectangle -clean -elevated temperature of immediate area	-no discoloration -small, white, raised nodule in center of area -elevated temperature of immediate area
gas exposed day 3	-smooth, clean, dark gray edges -small raised granular pink area in center of wound -elevated temperature of immediate area	--	-no discoloration -slight depression where biopsy was removed	--

Direct Effects of Gasoline Exposure on Wound Healing

During the time the incisions of BLM-B were being infused with crude oil or saline, thermographic records were made to monitor the reaction. Throughout the 64 minute observation period, the oil-infused wound was 1° to 2°C cooler than the saline-washed control (Table 6.1:8). This difference probably reflected evaporative cooling.

At the termination of the infusion period, the oil contaminated incision had stopped bleeding, while the saline-rinsed wound had not. By day 4, the edges of the oil-infused incision were in apposition, somewhat further advanced than the saline control.

In dolphin BLM-C, in which the incisions were extensively biopsied, there were no observable differences in the appearances between the non-exposed, and oil-contaminated wounds (Figure 6.1:21). Both remained clean and were closed by the fifthteenth day.

The edges of each biopsy wound appeared black, clean, and smooth for the first day (Figure 6.1:21 A). The base of each wound developed a firm, yellow to cream-colored core on the second day, which persisted until the wound had healed completely (Figure 6.1:21 B, C, D) by the tenth day. A pale gray halo surrounded the biopsy wounds. There was a gradual reduction in the size of the yellow plug, accompanied by a decrease in wound size over the course of healing. Although the wounds were closed by the tenth day, a black halo persisted around the biopsy scars for the 15 day period of observation (Figure 6.1:21 E).

Histologically, the healing patterns of the skin incisions were similar to those of other mammals (Harris and Youkey 1972, Peacock and vanWinkle 1976), with no observable differences related to oil. During the first 8 to 24 hours there was clot formation, subcutaneous hemorrhage,

Table 6.1:8 Maximum temperature of wounds in the skin of dolphin BLM-B. For 60 minutes, one wound was infused with crude oil, and the other with saline as a control. (Measured using thermography)

Time (min.) After Start of Wound Infusion	Temperature of Wound Area	
	Oil Infused	Saline Infused
11	34	36
23	33	34
64	33	34

epidermal thickening at the wound margin, dilation of blood vessels in the immediate area of the wound, and an immigration of inflammatory cells, primarily neutrophils (Figure 6.1:22 A and B). After 24 hours and for the remainder of the healing process, dolphin skin showed an additional reaction of marked hyaline degeneration of the cells of the stratum externum (Figure 6.1:22 C and D). By day 2 to day 3, the clot had become well-organized and filled with neutrophils. The cells of the stratum intermedium appeared to be undergoing an accelerated maturation and assumed the characteristics of the stratum externum still well within a few cell layers of the germinal layer compared to the normal maturation process which requires a passage of about 50 cell layers before this transition occurs. By day 4 and day 5 the proliferating surface epithelium had begun to migrate down over the denuded dermis where a bed of granulation tissue had begun to form (Figure 6.1:23). By day 16, there was complete regeneration of the epithelium at the wound site, though some hyaline degeneration persisted in the cells of the stratum externum, and there were numerous maturing fibroblasts in the dermis (Figure 6.1:24).

Figure 6.1:21

The progression of healing of 2 incisions made with a scalpel, on the back of Tursiops truncatus, BLM-C is shown in photos A to E. The incision on the right served as a control; that on the left was infused with West Texas crude oil for 30 minutes after which it was rinsed with sea water.

Figure 6.1:21 A

All cut edges appeared black, clean, and smooth 4 hours following oil contamination.

Figure 6.1:21 B

Biopsy wounds exhibited a firm, cream-colored core on day 4. The biopsy-free portion of each incision was black and clean. All wounds were surrounded by a halo of progressively lighter gray.

Figure 6.1:21 C and D

There was a gradual reduction in the sizes of both the yellow plugs and wound opening on days 5 and 6. Biopsy-free portions of both incisions were black and clean.

Figure 6.1:21 E

By day 15 both the incisions and biopsy wounds were closed. A dark black halo persisted around the wounds.

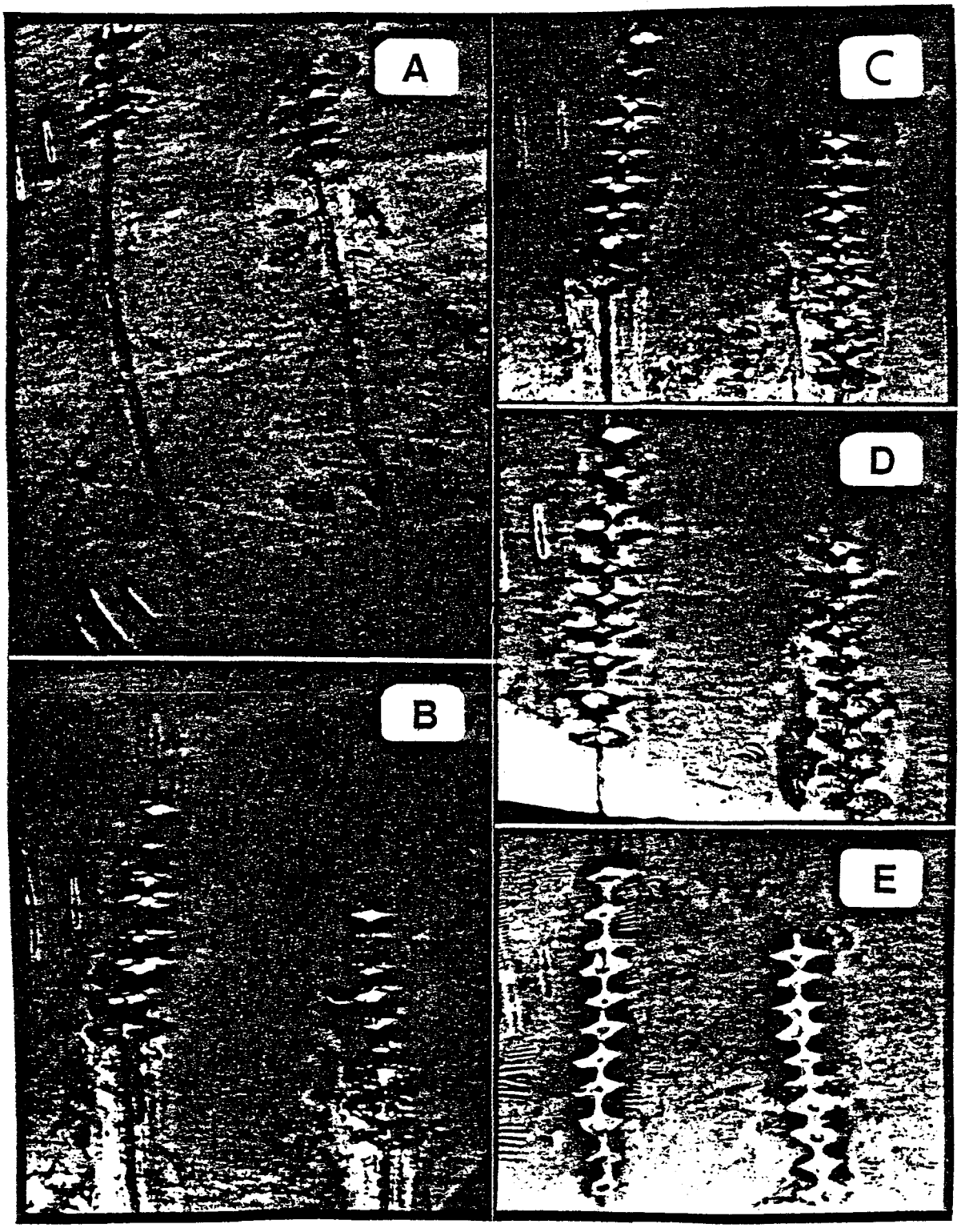


Figure 6.1:21 A-E

Figure 6.1:22 A

Biopsy sample of an oil contaminated wound site, 8 hours after the incision was made in the skin of dolphin BLM-C. The wound edge has retracted and the epidermis is thickened and darker in appearance at the shoulders of the wound (arrow). A clot (C) had formed in the wound. H and E x 100

Figure 6.1:22 B

Higher magnification of oil contaminated wound illustrating a fibrin clot with some red blood cells (arrow) and polymorphic neutrophils (open arrow head). H and E x 500

Figure 6.1:22 C

Two days after the control (non-contaminated) incision was made, the wound edge is still retracted and many of the more superficial cells at the wound edge are necrotic and have begun to slough. The dermal papillae immediately adjacent to the wound are infiltrated with inflammatory cells. However, the dermis immediately beneath the wound appears quite normal. There is marked hyaline degeneration of the cells of the upper stratum intermedium and the stratum externum. H and E x 150

Figure 6.1:22 D

High power photomicrograph of non-contaminated wound, illustrating the extensive hyaline degeneration of the upper layers of epithelial cells. H and E x 450

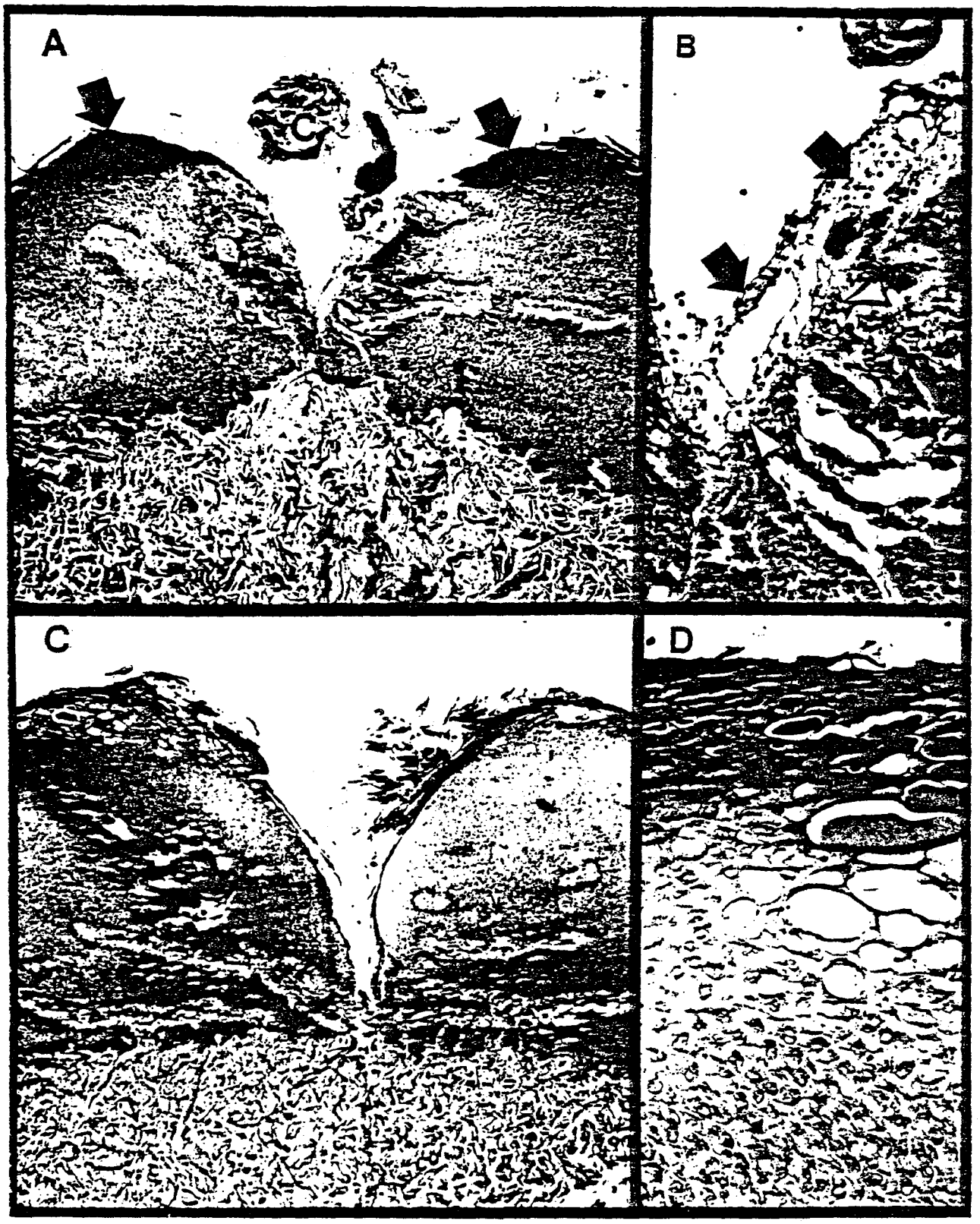


Figure 6.1:22 A-D

Figure 6.1:23 A

Bed of granulation tissue (G) in the dermis (D) underlying a 4-day-old wound. H and E x 400

Figure 6.1:23 B

Epithelial cells (E) migrating over denuded dermis in a 4-day-old wound. Note the presence of small numbers of inflammatory cells (arrows) in the dermis. H and E x 1,600

Figure 6.1:23 A

Bed of granulation tissue (G) in the dermis (D) underlying a 4-day-old wound. H and E x 400

Figure 6.1:23 B

Epithelial cells (E) migrating over denuded dermis in a 4-day-old wound. Note the presence of small numbers of inflammatory cells (arrows) in the dermis. H and E x 1,600

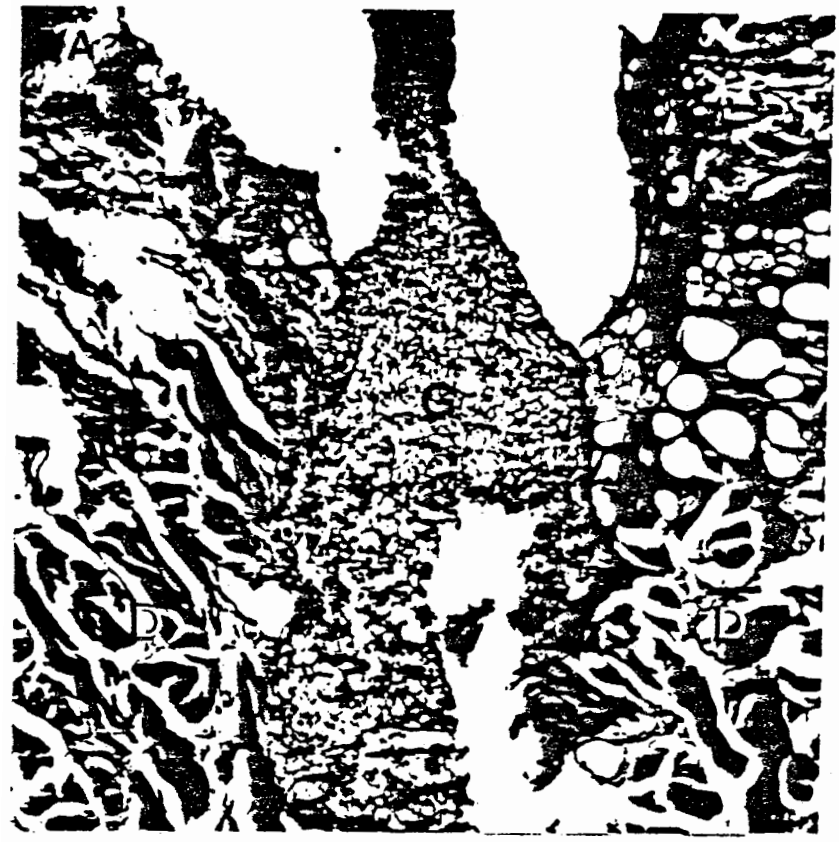


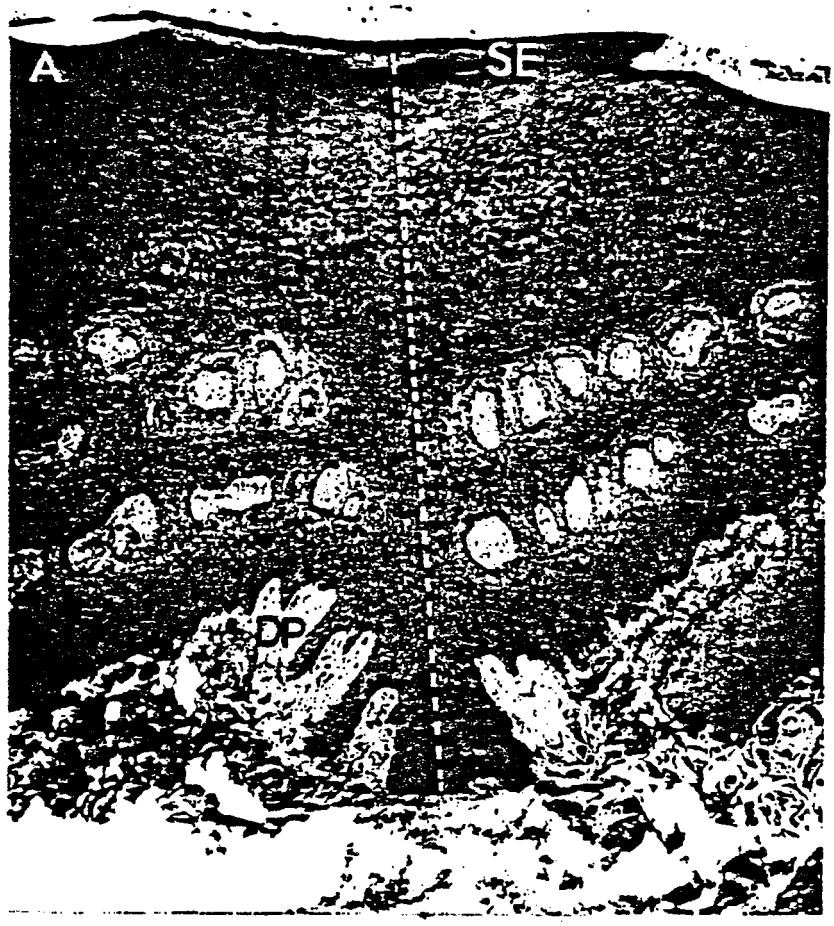
Figure 6.1:23 A and B

Figure 6.1:24 A

Low power photomicrograph of a 16-day-old wound (dotted line) which shows complete regeneration of the epithelium at the wound site. There is still some hyaline degeneration of the cells of the stratum externum (SE). The dermal papillae (DP) immediately adjacent to the wound contain increased numbers of maturing fibroblasts. H and E x 150

Figure 6.1:24 B

High power photomicrograph of the dermal papillae adjust to the wound site. There are numerous maturing fibroblasts (arrow) characteristic of early scar formation. H and E x 450



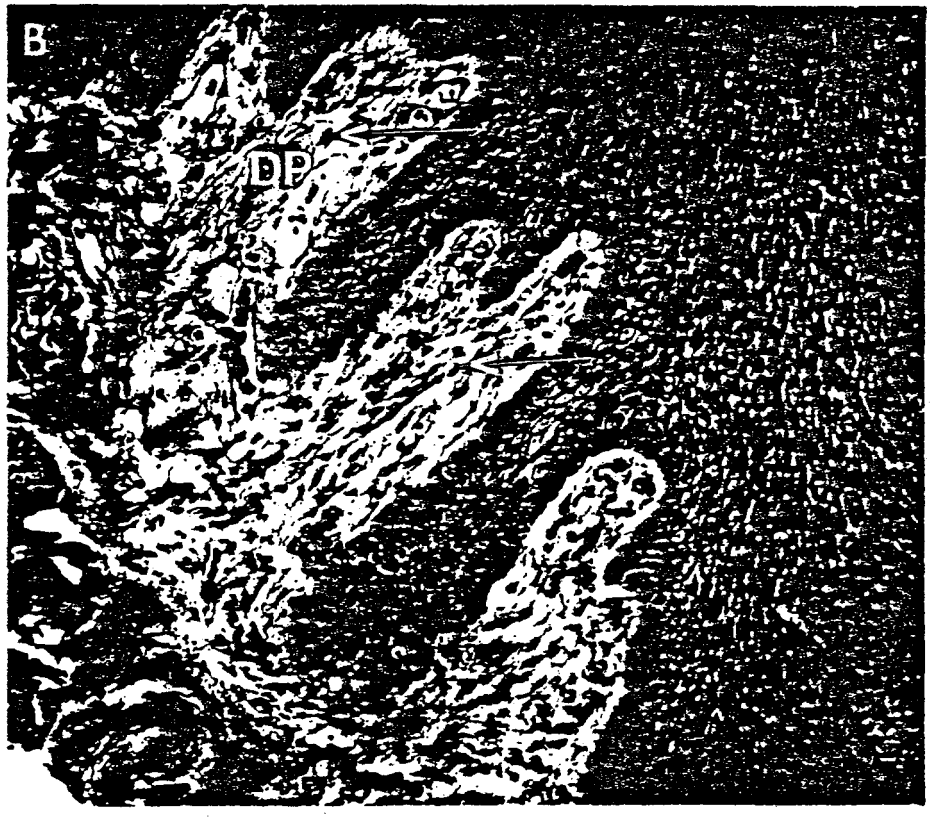


Figure 6.1:24 A and B

Surface Contact Studies on Free-Ranging Cetaceans

Introduction

During the course of this study, an opportunity arose to perform *in vitro* surface contact studies on a harbor porpoise, *Phocoena phocoena* as part of a Canadian government-sponsored research program in the Bay of Fundy, and a stranded sperm whale, *Physeter catodon*. We undertook these studies to expand our understanding of cetacean skin and its response to petroleum hydrocarbons.

Materials and Methods

In August 1980, the harbor porpoise was trapped in a herring weir, captured and euthanized. Pieces of skin 5 cm x 5 cm square and 2 cm thick were placed in 500 ml of fresh water, salt water, crude oil, or lead-free gasoline. Samples were taken from the outer edge of each piece at 30 min., and 1, 2, 4, 8, 14.5, and 35 hours for histological and ultrastructural examination. Samples were prepared and examined as described elsewhere in this report.

On August 13, 1981 a male sperm whale, *Physeter catodon*, stranded in Brit Bay on the northeast coast of Newfoundland. On August 15, the 53 foot, 25-year-old[1] whale was listing toward its right side in 3 m of water. An area of the left flank, just posterior to the flipper, approximately 3 m long and 1 m wide was high enough out of the water to allow us to perform contact studies. Three, 10 cm suction cups[2] containing

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[1] Aged by counting dentinal tooth layers (K. Hay, Can. Dept. Fisheries and Oceans, St. John's, Newfoundland).

[2] Canadian Tire Corporation

sponges soaked either in lead-free gasoline, crude oil (Inter-Provincial) or sea water were placed on the left flank of the whale. These were left in place for one hour, after which wedge-shaped biopsies approximately 3 cm long, 1/2 cm wide, and 1 cm deep were taken from the contact sites and adjacent nonexposed skin.

The suction cups were repositioned, and left in place overnight. When they could be retrieved, 17 hours later, the whale had been dead for 5 to 10 hours. Nevertheless, biopsies were taken because of the rare opportunity and unusual circumstances which this study presented.

Results and Discussion

Samples of harbor porpoise skin placed in fresh water, salt water, and crude oil showed rapidly progressing autolysis after 1 hour. Samples in gasoline showed early autolysis by 30 minutes but the autolysis did not progress. After 35 hours in gasoline, there appeared to be some fixation of tissue, as evidenced by intact nuclei and outer cell membranes. Most notable was the marked loss of intracellular lipid in skin exposed to gasoline for comparisons between 35 hours (Figure 6.1:25).

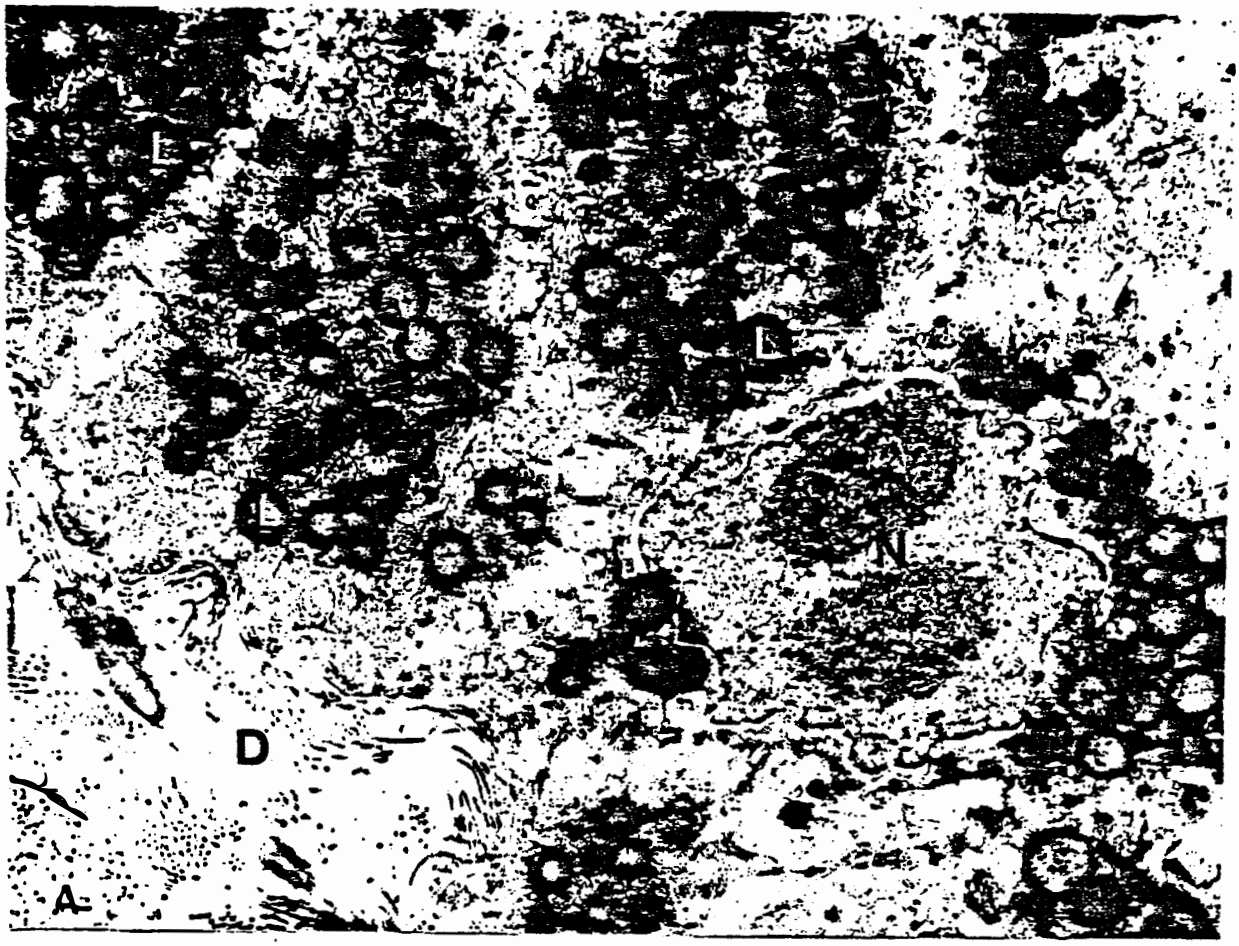
In the sperm whale, after the suction cups had been in place for 60 min., the edges of the contact sites were slightly (1-2 mm) indented. The site in contact with gasoline was pale in relation to non-exposed skin. There were no observable changes in the skin exposed to crude oil or sea water. Seventeen hours after the exposures, all three contact sites were normal in appearance. Histologically, there were no apparent lesions in any of the test sites following 60 min. exposure.

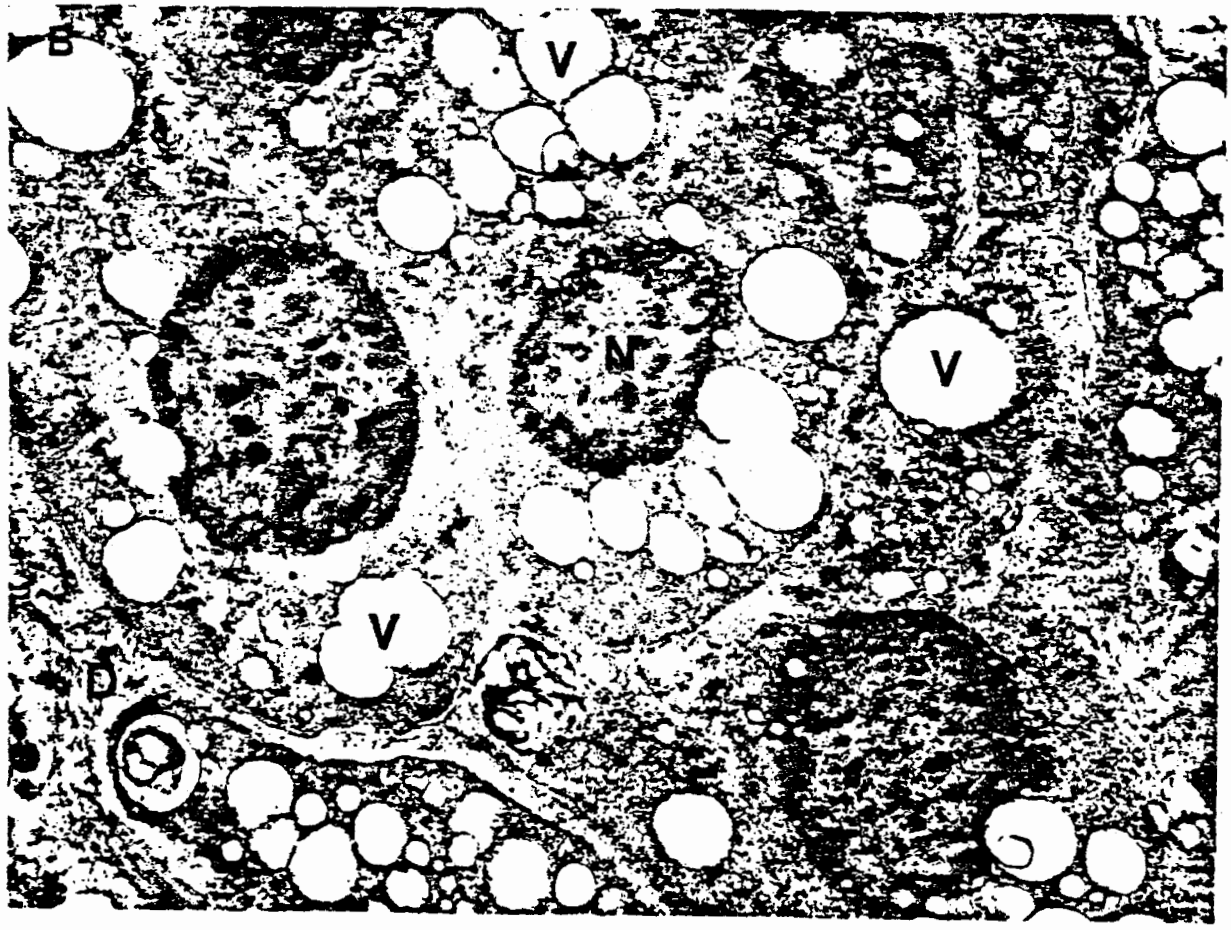
Figure 6.1:25 A

Normal stratum germinativum and the underlying dermis (D) from fresh skin of a Phocoena phocoena. Note the large number of intracellular lipid droplets (L). x 9,000

Figure 6.1:25 B

Stratum germinativum and the underlying dermis following 35 hours in vitro exposure to lead-free gasoline. The lipid has been dissolved away leaving clear vacuoles (V). x 9,000


L
L
L
N
D
A


B
V
N
V
V
D

The site exposed to sea water for 17 hours had an indented margin. The center of the contact area had a normal, though slightly wrinkled epidermis (Figure 6.1:26 A). Histologically, the epidermis at the control site had a few dead cells with pyknotic nuclei in the outer region of the stratum intermedium (Figure 6.1:26 B). The vessels of the underlying dermal papillae appeared mildly congested. Non-exposed skin immediately adjacent to the exposure site appeared normal.

The outer surface of the skin exposed to crude oil for 17 hours was soft but the uppermost layer still appeared to be intact (Figure 6.1:26 C). Histologically, there was pyknosis of several cell nuclei of the stratum intermedium. More cells were affected than at the control site (Figure 6.1:26 D). Some separation (acantholysis) of adjacent cells of the stratum intermedium had also occurred. These changes were restricted to the outer half of the stratum intermedium.

The site exposed to gasoline for 17 hours showed dramatic changes. The original skin surface could not be defined and the upper 1/2 to 1/3 of the epidermis was pale gray and had the consistency of thick paste (Figure 6.1:26 E). Histologically, the stratum externum and the upper 1/2 to 2/3 of the stratum intermedium were lost. There was marked pyknosis and separation of the remaining cells of the stratum intermedium distal to the dermal papillae (Figure 6.1:26 F). The stratum intermedium immediately adjacent to the dermal papillae and the stratum germinativum appeared normal.

Figure 6.1:26 A

On removal of the suction cup containing salt water, the epidermis was intact but wrinkled. There was a depression in the skin around the circumference of the suction cup.

Figure 6.1:26 B

Microscopically, there was some evidence of necrosis under the control suction cup, indicated by the presence of some pyknotic nuclei (arrows) in the upper portion of the stratum intermedium. H and E x 425.

Figure 6.1:26 C

After 17 hours exposure to crude oil, the epithelium was soft but intact, and the circumference of the suction cup application site was indented.

Figure 6.1:26 D

Photomicrograph of crude oil contact site, illustrating some pyknotic nuclei similar to those observed in the salt water contact site. H and E x 425

Figure 6.1:26 E

After 17 hours exposure to lead-free gasoline, the central region of the contact site was depressed and had the consistency of a thick gray paste, and the epithelium was lost.

Figure 6.1:26 F

Photomicrograph of lead-free gasoline contact site showing marked necrosis and acantholysis of the stratum intermedium. H and E x 425

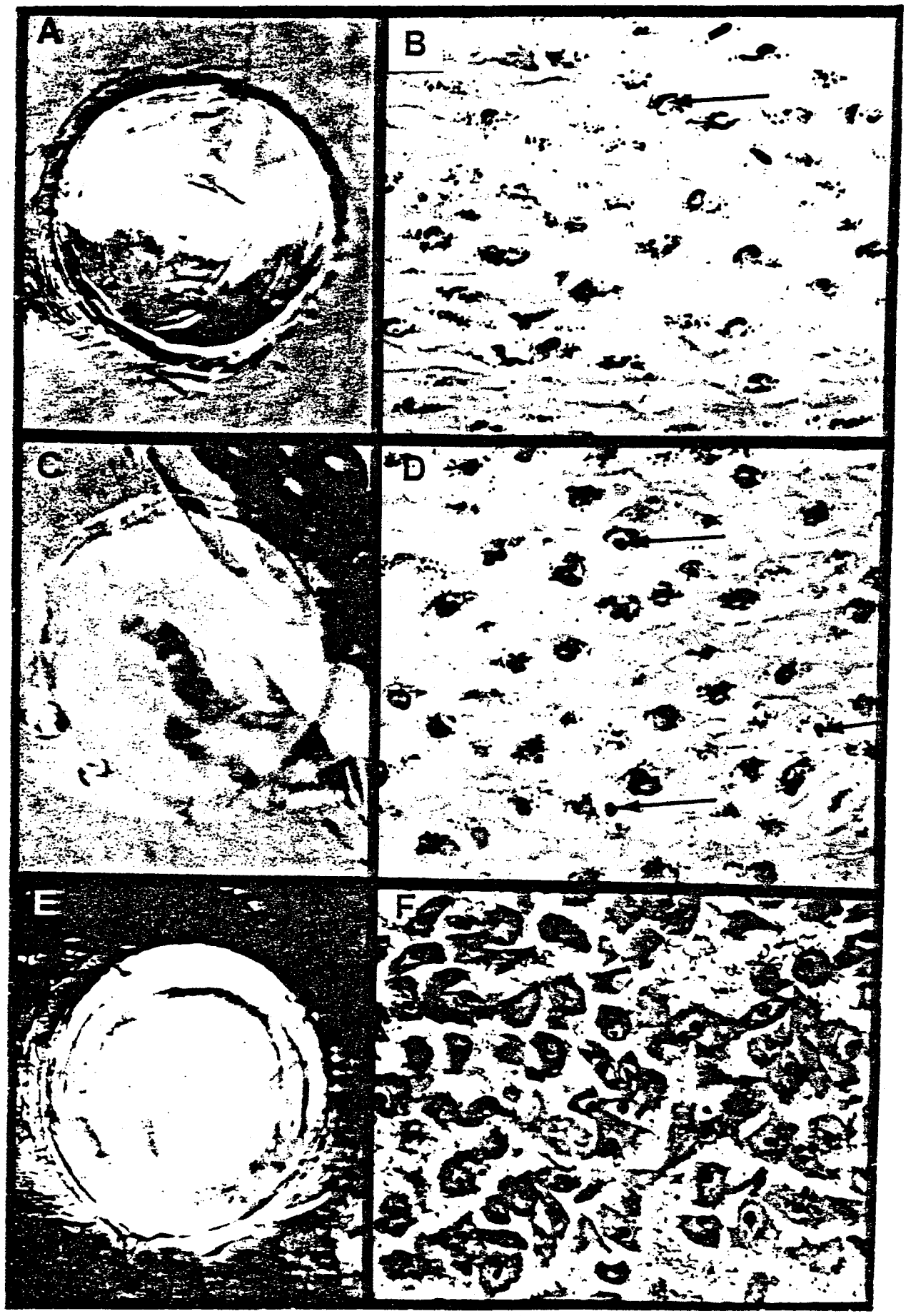


Figure 6.1:26 A-F

The whale died during the course of the contact study. Yet the histological changes noted in the epidermis exposed to gasoline are noteworthy in that they are indicative of damage to living cells, and not postmortem autolysis. This interpretation is supported by the fact that skin exposed to crude oil was only mildly affected, while control and non-exposed samples were relatively unaffected. Furthermore, the truly devitalized skin of the harbor porpoise showed no such loss of integrity after 35 hours, and in fact, evidenced some degree of preservation.

These field studies though not performed under ideal circumstances, provided us with 2 important clues into the nature of petroleum-induced skin damage. Long-term exposure to gasoline can lead to severe necrosis of the medial and superficial layers of the epidermis. As the stratum germinativum and underlying dermis appear to remain functional, even this degree of damage seems to be reversible. The brief study on Phocoena was notable in demonstrating that a primary effect of gasoline exposure may be delipidation of epithelial cells. Though the function of the lipids is not fully understood, their removal by volatile hydrocarbons might have far reaching effects on physical (Essapian 1955), biochemical (see Section 6.2, this report), and antimicrobial (Waldorf and Vedros 1978) properties of skin.

References

(1) Dutton, W.F. 1934. Petroleum dermatitis. Med. Rec. 140: 550-552.

(2) Essapian, F.S. 1955. Speed induced skin folds in the bottlenosed porpoise, Tursiops truncatus. Breviora, Mus. Comp. Zool., Cambridge MA.

(3) Harris, D.R. and J.R. Youkey. 1972. Evaluating the effects of hemostatic agents on the healing of superficial wounds. In Epidermal Wound Healing (H.I. Mailbach and D.T. Rovee, Ed.), Yearbook Medical Publishers Inc., Chicago.

(4) Harrison, R.J. and K.W. Thurley. 1974. Structure of the epidermis of Tursiops, Delphinus, Orcinus, and Phocoena. In Functional Anatomy of Marine Mammals, Vol. 2. (R.J. Harrison Ed.) Academic Press, New York, NY.

(5) Kleinenberg, S.E., A.V. Yablokov, B.M. Bel'kovich and M.N. Tarasevich. 1969. Beluga (Delphinapterus leucas) investigation of the species. Israel Program of Sci. Transl., Jerusalem.

(6) Kooyman, G.L., R.L. Gentry and W.B. McAllister. 1976. Physiological impact of oil on pinnipeds. Northwest Fish. Center Processed Report. U.S. Dept. of Commerce, NOAA, NMFS, Seattle, WA. 23 pp.

(7) Kooyman, G.L., R.W. Davis and M.A. Castellini. 1977. Thermal conductance of immersed pinniped and sea otter pelts before and after oiling with Prudhoe Bay crude. In Fate and Effects of Petroleum Hydrocarbons in Marine Ecosystems and Organisms. (D.A. Wolfe, Ed.), Pergamon Press, Oxford. pp. 151-157.

(8) Ling, J.K. 1974. The integument of marine mammals. In Functional Anatomy of Marine Mammals (R.J. Harrison, Ed.), Academic Press. 2: 1-44.

(9) Lupulescu, A.P., D.J. Birmingham and H. Pinkus. 1973. An electron microscopic study of human epidermis after acetone and kerosene administration. J. Invest. Derm., 60: 33-45.

(10) Palmer, E. and G. Weddell. 1964. The relationship between structure innervation and function of the skin of the bottlenose dolphin (Tursiops truncatus). Proc. Zool. Soc. Lond. 143: 553-568.

(11) Peacock, E.E. and W. vanWinkle. 1976. Wound Repair. W.B. Saunders Co., Toronto, Ontario.

(12) Simpson, J.G. and M.B. Gardner. 1972. Comparative microscopic anatomy of selected marine mammals. In Mammals of the Sea: Biology and Medicine. (S. Ridgway Ed.), Charles C. Thomas, Springfield, VA.

(13) Sokolov, V. and M. Kalishnikova. 1971. The ultrastructure of epidermal cells in Phocoena phocoena. In Investigation on Cetacea. Vol. 3, Part 2. (G. Pilleri Ed.). pp. 194-199.

(14) Sperman, R.I.C. 1972. The epidermal stratum corneum of the whale. J. Anat. 113: 373-381.

(15) Threadgold, L.T. 1976. The Ultrastructure of the Animal Cell. Second Edition, Pergamon Press, Oxford. pp. 269-290.

(16) Trump, B.R. and A.U. Arstila. 1975. Cell membranes and disease processes. In Pathobiology of Cell Membranes. Vol. 1. (B.R. Trump and A.U. Arstila Ed.). Academic Press, New York, NY.

(17) Villaume, J., P. Walter, R. Levine, A. Craigmill and H. Schwartz. 1976. Petroleum distillates. NTIS Report No. PB-282-404-3ST.

(18) Waldorf, A. and N.A. Vedros. 1978. Northern fur seal, Callorhinus ursinus, the skin and fatty acids as a natural barrier to fungal penetration. Aquatic Mammals 6 (1): 77-89.

(19) Williams, T.D. 1978. Chemical immobilization, baseline hematological parameters and oil contamination in the sea otter. U.S. Marine Mammal Commission Report No. MMC-77/06. NTIS, Springfield, VA. 27 pp.

(20) Zelickson, A.S. 1967. Ultrastructure of Normal and Abnormal Skin. Lea and Febiger, Philadelphia, PA.

6.2 Biochemical and Physiological Effects of Oil on Cetacean Skin

Cetacean skin is not only unique in its morphology, but has unusual chemical and biochemical characteristics. Previous studies have demonstrated that the epidermal cells are surprisingly rich in enzymes (Geraci and St. Aubin 1979) and vitamin C (St. Aubin and Geraci 1980). The reason(s) for this activity is not fully understood, but it suggests that the epidermal cells must maintain considerable metabolic function during the course of their maturation and displacement toward the surface of the skin. The presence of such biochemical machinery also suggests that the cells might be vulnerable to any toxic insult which inhibits its enzymatic pathways. We selected three constituents or metabolic processes in cetacean skin which might be affected by exposure to oil, and used them as sensitive indicators of subtle damage not detectable microscopically. Specifically, we studied the incorporation of fatty acids into phospholipids, the activity of creatine kinase, and the rate of aerobic metabolism in cetacean skin. Baseline data were determined in samples taken from freshly dead stranded or captive animals, and from biopsies of live cetaceans. Both *in vivo* and *in vitro* exposures were used to assess the effects of oil on these biochemical characteristics.

Sample Acquisition

Tissue samples were removed from a 1-year-old *Tursiops* (MH-79-180-Tt) and a newborn *Tursiops* (MH-81-18-Tt) immediately after they died at the New England Aquarium. Portions of skin and liver were placed on ice and analyzed for phospholipid synthesis within 24 hours. Tissues for CK were placed on ice for up to 2 hours, before they were frozen at -20°C. They were stored for up to 30 days at -70°C prior to analysis.

A Tursiops (Tt 25) which died at the Montreal Aquarium provided skin and liver for studies on oxygen consumption. Samples were stored on ice for 24 hours prior to analysis.

A live Risso's dolphin, Grampus griseus, held at the New England Aquarium was exposed for 45 minutes to lead-free gasoline, using a S.C. dish (see Section 6.1, this report). Biopsies of test and control areas were placed in a plastic vial and frozen immediately using dry ice. Samples were analyzed for CK after storage for up to 30 days at -70°C. After a 17 minute exposure to a mixture of gasoline and oil taken from a 2-cycle generator, the skin of the Grampus was sampled for studies on phospholipid synthesis. The biopsies were placed on ice immediately and processed within 24 hours.

Liver and skin were obtained for tissue respiration studies from a freshly killed harbor porpoise, Phocoena phocoena. The study was carried out at Deer Island, New Brunswick, in conjunction with a Canadian government investigation into the biology and population dynamics of this species in the Bay of Fundy. Tissues were removed and placed on ice 50 minutes after the animal was killed by gunshot. Processing for measurement of oxygen consumption was begun immediately.

Metabolic activity was measured in skin samples from two cetaceans which died after stranding along the New England coast. Tissues from a Phocoena (MH-80-440-Pp) and a pigmy sperm whale, Kogia breviceps, (MH-80-394-Kb) were placed on ice, (frozen) and transported to the laboratory where they were analyzed within 24 hours.

Creatine Kinase Analysis

Tissue homogenates were prepared using the method of Herzfeld and Greengard (1971). Tissue samples weighing 10-20 mg were placed immediately in 4 ml of cold phosphate buffered saline (PBS, pH 7.5-7.6). The tissues were homogenized with a Polytron blender[1] at approximately 15000 rpm for 15-20 sec. at 22°C. A second homogenization using a glass homogenizer, assured all skin tissue entered solution. Triton X-100[2] was then added to a final concentration of 0.5% v/v, and the samples placed at 4°C, for 30 min. The fibrous elements were removed by centrifugation at 1110 x g for 10 min. at 25°C. The supernatants were decanted and stored at 4°C, for use in both total CK and isoenzyme activity determinations.

Total CK activity of the supernatants was determined within 4 hours, using the BMC test kit 124184,[3] UV method. All solutions, including the supernatant, were brought to room temperature (22°C). A 0.05 ml volume of supernatant was added to 1.05 ml of the reagent solution, which contained a triethanolamine buffer at pH 7.0, enzymes, coenzyme, and a creatine phosphate substrate. The solution was covered and mixed by gentle inversion, then allowed to incubate for 5 minutes at 25°C. The CK activity was read on a Coleman 55 Spectrophotometer[4] as a function of change in absorbance at 340 nm. Enzyme activity is reported in milli-international units (mU),

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1 Brinkmann Instruments, Rexdale, Ontario, Canada
2 J.T. Baker Chemical Co., Phillipsburg, NJ
3 Boehringer Mannheim, Mannheim, Germany
4 Perkin-Elmer, Maywood, IL

defined as the conversion of one millimicromole of substate per minute.

A lyophilized serum preparation, Precinorm E[5], reconstituted with distilled water was treated as a sample. It served as a control for enzyme activity, and for determination of day-to-day variability.

Isoenzyme separation followed the method of Mercer (1974). Separation columns were prepared by suspending an anion-exchanger, DEAE-Sephadex A-50[6] in distilled water, then filling a 12.5 Pasteur pipet with the slurry. A 1.0 ml volume of test supernatant, was introduced to the column at 25°C. The CK isoenzymes bound to the DEAE-Sephadex beads, and the eluent was collected in a vial.

By subsequently introducing 1.0 ml volumes of tris-hydrochloride buffered sodium chloride with incresing chloride concentrations, the isoenzymes were selectively replaced on the column according to their binding capacity. Three rinsings with each of 3 sodium chloride solutions (0.1 M, 0.2 M, 0.3 M, respectively) ensured complete collection of each isoenzyme with no carry-over of one isoenzyme to the next. Eluents were collected from the column after each washing in separate vials numbered 2 to 10. Vials 1, 2, 3, and 4 contained the MM isoenzyme, vials 5, 6, and 7 contained the MB isoenzyme, and vials 8, 9 and 10 contained the BB isoenzyme. Total elution took approximately 30 min. The 10 eluents were each assayed for CK activity according to the method previously described.

Muscle and heart tissues were analyzed for CK and CK isoenzymes to assure that the system was accurate and sensitive enough to separate the MM and MB isoenzymes.

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[5] Boehringer Mannheim, Mannheim, Germany
[6] Sigma Chemical Co., St. Louis, MO

Measurement of Phospholipid Synthesis

The epidermis was removed from the dermis by slicing with a razor blade, avoiding as much as possible, the underlying blubber. The sample was homogenized at 0°C-4°C in a Polytron for 30 seconds at medium speed, then ground in 20 volumes of ice cold 0.25 M sucrose-50 mM Tris-HCl buffer, pH 7.4, in a ground glass homogenizer. The homogenate was centrifuged at 2100 x g for 10 minutes. The supernatant was used for assays, and an aliquot was removed for protein determination by the method of Lowry, et al. (1951).

Fatty acid incorporation assay was carried out in an incubation medium of 5 ml containing 50 mM Tris-HCl buffer, pH 7.4, 100 µg of the 2100 x g supernatant protein suspended in approximately 30 µl of 0.25 M Sucrose-50 mM Tris-HCl buffer, pH 7.4, and 50 nCi (0.835 nmoles, 64,000 CPM) of (Stearoyl--1-^{14}C) stearoyl CoA. Incubation was also carried out with (oleoyl--1-^{14}C) oleoyl CoA (50 nCi, 0.873 nmoles, 71,000 CPM) and 2-lyso phosphatidylcholine (1 µM) as substrates instead of stearoyl CoA. ^{14}C-fatty acyl CoA was added to initiate the reaction and incubation was carried out for 30 minutes at 25°C. The reaction was terminated by adding 9.5 ml of chloroform/methanol (2:1, v/v) and lipids were extracted according to the method described by Folch et al. (1957). One ml of the lipid extract was transferred to glass scintillation vials. The chloroform solvent was evaporated dry at 200°F, under a flow of nitrogen gas. To the residue, 15 ml of Amersham OCS cocktail was added and the amount of radioactivity was measured by a Searle Delta 300 liquid scintillation counter. The remaining 6 ml lipid extract was evaporated dry, reconstituted in 50 µl of chloroform/methanol (2:1, v/v) and spotted onto Anasil HF or Whatman K5 TLC plates. The vials were rinsed with 50 µl of chloroform/methanol and spotted again. The plates were developed for 3.5 hours in a TLC chamber containing chloroform/absolute ethanol/distilled H_2O/triethylamine (60:68:16:70, v/v). After

development, the solvent was evaporated off and the plates were sprayed with a solution of 2', 7'-dichlorofluorescein in absolute ethanol/methanol (70:30, v/v). The plates were reviewed under UV light and the phospholipid bands were identified. Each band was scraped from the plate and transferred to scintillation vials. To each, 15 ml of OCS was added and the amount of radioactivity present was measured.

Measurement of Metabolic Activity

Metabolic activity of cetacean epidermis was measured using the direct method of Warburg, as described by Umbreit et al. (1972) and outlined in the Gilson Differential Respirometer manual[7]. Sections of epidermis were separated from the dermis and sliced to approximately 1 mm thickness. They were then blotted dry and weighed. A two gram sample was diced with scissors and added to a chilled test tube containing the suspending medium. The suspension was homogenized in a crushed ice bath with a polytron Ultrasonic disintegrator[8] at maximum speed for 20 seconds until the skin was reduced to small particles. Initially, the homogenate was filtered through cheesecloth, and the filtrate and the remaining solid portion were analyzed separately. The results of this procedure indicated that the solid portion of the homogenate must be included for activity to be measured.

One milliliter of the homogenate was pipetted into each of four 15 ml respirometer flasks containing 2 ml of suspending medium. These flasks are designed with a sidearm which contain 0.5 ml of substrate that can be discharged into the flask by gently angling the flask.

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[7] Mandel Scientific, Montreal, Quebec, Canada
[8] Brinkmann Instruments Ltd., Rexdale, Ontario, Canada

The most satisfactory results were obtained using Krebs-Ringer phosphate as the suspending medium and 0.25 M succinate as the substrate. To absorb CO_2, 0.2 ml of 20% potassium hydroxide was added onto a folded paper wick in the center well of the flask. The top, and all glass joints were lubricated with petrolatum, and the flasks were attached to the shaking rack of the respirometer and lowered into the 30°C waterbath to equilibrate for 20 minutes. The system was closed and readings were taken at 0, 10, and 20 minutes to measure the endogenous activity - that is, the activity of the tissue preparation without adding substrate. After 20 minutes the substrate in the sidearm was tipped into the flask, and readings were taken every 10 minutes for 1 hour. The zero time reading was then subtracted from the final reading to determine the total volume of oxygen consumed. From the known quantity of tissue, reaction time, and oxygen consumption, the respiratory activity of the preparation was calculated in terms of μl O_2 consumed/mg tissue/hour.

Tissues from the Phocoena collected in the Bay of Fundy were homogenized in ice cold 0.25 M sucrose using a Polytron sonicator at full speed for 10 sec. One ml of the homogenate was added to Krebs-Ringer phosphate and the standard procedure was followed, incubating the flasks at 30°C. A separate sample of skin homogenate was added to sea water as the suspending medium, and incubated at 14°C to determine whether activity would be greater if the tissue were incubated under the same conditions to which Phocoena skin is normally exposed.

Rabbit liver was used as a control for most of the studies, and also in a study of the stability of the enzyme systems under various storage conditions. Samples were analyzed while fresh, and after being stored for up to 24 hours at 22°C, 7 days at 4°C, and 37 days at -20°C and -70°C.

Results and Discussion

Creatine Kinase

The total CK activity of Tursiops and Grampus skin (Table 6.2:1) was within the range previously reported for cetaceans (Geraci and St. Aubin 1979). Creatine kinase is an enzyme composed of 2 subunits, designated as M and B. These combine to form one of three dimeric units, MM, MB, or BB. Generally, the MM unit is found principally in skeletal muscle, the BB unit in brain, and the MB hybrid in cardiac muscle. The isoenzyme patterns of the cetacean skin samples show activity predominantly in the MM or muscle fraction. Exposure to lead-free gasoline in vivo had no effect on the activity CK in epidermal cells.

Phospholipid Synthesis

Exposure to lead-free gasoline in vivo affected the incorporation of labelled stearic acid into some phospholipids (Table 6.2:2). Incorporation of labels into sphingomyelin, phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine and neutral lipids was less than in the control sample. This reduction in activity could have been due to the physical destruction of epidermal cells by crude oil directly affecting the synthesis of lipids. There is also the possibility that some components of crude oil inhibited certain steps in the synthesis of lipids. There was some increase in phosphatidylserine production. This may have resulted from increased availability of substrates due to a reduction in activity in other pathways of lipid synthesis that shared those substrates as common precursors. Incorporation rates of labelled oleic acid into lipids were affected similarly in oil-treated skin (Table 6.2:3).

Table 6.2:1 Creatine Kinase (CK) isoenzyme activity in selected cetacean tissues, including skin from *Grampus griseus* before and after exposure to lead-free gasoline

Species Identification Code	Tissue	Total CK activity (mU/mg fresh wt)	Isoenzyme activity (mU/mg fresh wt) MM[1]	MB[2]	BB[3]	Fractional activity (%) MM	MB	BB
Tursiops truncatus								
MH-81-19 Tt	skin	40.05	23.7	6.7	12	60	16	24
MH-81-19 Tt	skin	29.63	20.1	3.0	3.5	75	11	13
MH-79-180 Tt	skin	32.28	24.2	0	0	100	0	0
MH-79-180 Tt	skeletal muscle	1343.60	1520	43.2	0	97	3	0
MH-79-180 Tt	heart	148.90	175.3	46.3	0	80	20	0
Grampus griseus								
SC-Gg-10C	skin	11.33	13.2	0	0	100	0	0
45 min. exposure to lead-free gasoline								
5 min. post exposure	skin	18.00	14.8	0	0	100	0	0
2 days post exposure	skin	16.80	16.8	0	0	100	0	0
4 days post exposure	skin	10.66	7.8	0	0	100	0	0

[1] skeletal muscle isoenzyme of CK

[2] cardiac muscle isoenzyme of CK

[3] brain isoenzyme of CK

Table 6.2:2 Incorporation of ^{14}C-stearic acid *in vivo* into lipids of oil treated dolphin skin.

CPM-background ± SE Lipid	Control	Oil Treated	% of Control
origin	659 ± 110	892 ± 52	135 ± 8
sphingomyelin	209 ± 56	169 ± 23	81 ± 11
phosphatidylcholine	1457 ± 27	972 ± 92	67 ± 6
phosphatidylserine	4723 ± 174	5116 ± 12	108 ± 1
phosphatidylinositol	244 ± 6	209 ± 30	86 ± 12
phosphatidylethanolamine	343 ± 33	295	86
phosphatidylglycerol	4600 ± 183	4577	99
fatty acid	6525 ± 604	8907 ± 600	137 ± 9
neutral lipids	3619 ± 304	1842 ± 166	51 ± 5

Table 6.2:3 Incorporation of ^{14}C-oleic acid into lipids of cetacean skin exposed *in vivo* to petroleum hydrocarbons. 1 µM of 2-lyso phosphatidylcholine was included in the incubation medium.

CPM-background ± SE Lipid	Control	Oil Treated	% of Control
origin	805 ± 80	971 ± 181	121 ± 22
sphingomyelin	301 ± 37	162 ± 36	54 ± 12
phosphatidylcholine	6707 ± 176	2239 ± 10	33 ± 1
phosphatidylserine	4798 ± 259	5987 ± 94	125 ± 2
phosphatidylinositol	619 ± 13	645 ± 30	104 ± 5
phosphatidylethanolamine	489 ± 31	313 ± 8	64 ± 2
phosphatidylglycerol	2415 ± 304	1858 ± 174	77 ± 7
fatty acid	11760 ± 166	13139 ± 73	112 ± 1
neutral lipids	6343 ± 152	3092 ± 135	49 ± 2

When the 2100 x g supernatant of homogenized skin was incubated in the presence of 100 ppm of oil (in vitro study), a different pattern of reduced activity in lipids was observed (Table 6.2:4-6.2:7). Phosphatidylethanolamine and phosphatidylinositol contained less label when stearic acid was used as a substrate, and less sphingomyelin, phosphatidylethanolamine, and phosphatidylglycerol when oleic acid was used as substrate. Incorporation rates of label into neutral lipid and phosphatidylcholine molecules were not affected. This reduction in activity was apparent only when the skin supernatant was first incubated in the presence of the oil before the assays were carried out (Table 6.2:8).

These studies showed that in vivo and in vitro exposure to oil results in a depression of phospholipid synthesis. This defect may represent a biochemical expression of the ultrastructural changes in cell integrity that we observed following exposure to gasoline (see Section 6.1, this report). Alternatively, some component of the hydrocarbons may have interfered with one of the steps in the synthesis of phospholipids. In either case, the biochemical defect could jeopardize the integrity of cell membranes, with ultimate consequences dependent on the extent and reversibility of the damage.

Metabolic Activity

Studies on the metabolic activity were initially hampered by the availability of fresh skin specimens. Analyses performed on tissues which had been frozen did not yield satisfactory results. Using rabbit liver, we quantified the stability of the enzyme systems under different storage conditions.

Table 6.2:4 Incorporation of ^{14}C-stearic acid *in vitro* into lipids of *Tursiops* (MH-79-180-Tt) epidermal cells. 100 ppm of crude oil was included in the incubation medium. Pre-incubation was carried out for 5 minutes at 20°C.

CPM-background ± SE			
Lipid	Control	Oil Treated	% of Control
sphingomyelin	335 ± 5	387 ± 85	116 ± 25
phosphatidylcholine	2813 ± 25	3030 ± 2	108 ± 1
phosphatidylserine	1526 ± 27	1639 ± 11	107 ± 1
phosphatidylinositol	1195 ± 116	933 ± 56	78 ± 5
phosphatidylethanolamine	3125 ± 27	1356 ± 55	43 ± 2
phosphatidylglycerol	6899 ± 363	7719 ± 221	112 ± 3
fatty acid	13221 ± 485	13885 ± 305	105 ± 2
neutral lipids	3046 ± 243	3074 ± 4	101 ± 1

Table 6.2:5 Incorporation of ^{14}C-oleic acid *in vitro* into lipids of *Tursiops* (MH-79-180-Tt) epidermal cells. 100 ppm of crude oil and 1 µM of 2-lyso phosphatidylcholine were included in the incubation medium. Pre-incubation was carried out for 5 minutes at 20°C.

CPM-background ± SE			
Lipid	Control	Oil Treated	% of Control
sphingomyelin	405 ± 61	345 ± 51	85 ± 13
phosphatidylcholine	8974 ± 4	8980 ± 279	100 ± 3
phosphatidylserine	1324 ± 20	1545 ± 64	117 ± 5
phosphatidylinositol	2935 ± 441	2853 ± 526	97 ± 18
phosphatidylethanolamine	3008 ± 38	1626 ± 798	54 ± 27
phosphatidylglycerol	7236 ± 816	3904 ± 1518	54 ± 21
fatty acid	18594 ± 279	17403 ± 785	94 ± 4
neutral lipids	2790 ± 9	3469 ± 44	124 ± 4

Table 6.2:6 Incorporation of ^{14}C-stearic acid *in vitro* into lipids of *Tursiops* (MH-81-19-Tt) epidermal cells, in the presence of 100 ppm of crude oil. Pre-incubation was carried out for 30 minutes.

CPM-background ± SE			
Lipid	Control	Oil Treated	% of Control
sphingomyelin	206 ± 39	152 ± 37	74 ± 18
phosphatidylcholine	706 ± 41	589 ± 53	83 ± 8
phosphatidylserine	1811 ± 183	1851 ± 58	102 ± 3
phosphatidylinositol	202 ± 14	199 ± 6	99 ± 3
phosphatidylethanolamine	1053 ± 54	236 ± 15	22 ± 1
phosphatidylglycerol	3797 ± 107	3394 ± 138	89 ± 4
fatty acid	12320 ± 47	12340 ± 340	100 ± 3
neutral lipids	1476 ± 158	2105 ± 110	143 ± 7

Table 6.2:7 Incorporation of ^{14}C-oleic acid *in vitro* into lipids of *Tursiops* (MH-81-19-Tt) epidermal cells, in the presence of 100 ppm of crude oil and 1 µM of 2 lyso phosphatidylcholine. Pre-incubation was carried out for 30 minutes.

CPM-background ± SE			
Lipid	Control	Oil Treated	% of Control
sphingomyelin	218 ± 2	217 ± 32	100 ± 15
phosphatidylcholine	1753 ± 29	2044 ± 5	117 ± 1
phosphatidylserine	1998 ± 39	2183 ± 28	109 ± 1
phosphatidylinositol	385 ± 42	415 ± 5	108 ± 1
phosphatidylethanolamine	989 ± 16	679 ± 72	69 ± 7
phosphatidylglycerol	2232 ± 329	2407 ± 258	108 ± 12
fatty acid	26129 ± 645	22619 ± 480	87 ± 2
neutral lipids	2934 ± 214	4177 ± 506	142 ± 17

Table 6.2:3 Effects of pre-incubation on the incorporation of ^{14}C-oleic acid *in vitro* into lipids of *Tursiops* (MH-79-180-Tt) epidermal cells. 100 ppm of crude oil and 1 μM of 2-lyso phosphatidylcholine were included in the incubation medium.

CPM-background ± SE Lipid	Control	Oil Treated No Pre-Incubation	Oil Treated 30 Min. Pre-Incubation
sphingomyelin	670 ± 44	543 ± 149	451 ± 1
% of control	100%	81% ± 22%	67% ± 1%
phosphatidylcholine	13478 ± 1378	12648 ± 1264	15059 ± 89
% of control	100%	94% ± 9%	112% ± 1%
phosphatidylserine	1074 ± 13	1116 ± 82	1262 ± 13
% of control	100%	104% ± 8%	118% ± 1%
phosphatidylinositol	2178 ± 113	2150 ± 161	2060 ± 113
% of control	100%	99% ± 7%	95% ± 5%
phosphatidylethanolamine	2435 ± 142	2382 ± 207	1346 ± 2
% of control	100%	98% ± 9%	55% ± 1%
phosphatidylglycerol	2071 ± 198	1710 ± 249	1470 ± 121
% of control	100%	83% ± 12%	71% ± 6%
fatty acid	25979 ± 538	23382 ± 170	23770 ± 388
% of control	100%	90% ± 1%	91% ± 1%
neutral lipids	3649 ± 80	3300 ± 141	3415 ± 5
% of control	100%	90% ± 4%	94% ± 1%

Specimens stored at 22°C lost 58% activity within 2 hours, and 71% by 24 hours. Refrigerated samples (4°C) stored in phosphate buffered saline showed relatively stable activity for the first 24 hours. Thereafter, activity declined to 87-95% of the original activity after 48 hours, 68% by 72 hours, and approximately 60% by day 6.

Specimens frozen at -20°C lost at least 50% of their activity immediately, and thereafter gradually lost over 80% by 14 days (Table 6.2:9). Freezing at -70°C resulted in a 30% reduction in activity at 24 hours. Activity then remained stable at 40 to 50% of the fresh sample value, for up to 37 days.

It was thus concluded that specimens stored in any manner were less suitable for analysis than fresh tissue, and that material obtained from animals in the field should ideally be analyzed on location, immediately, or within 24 hours if stored on ice. The opportunity to perform such a study arose as part of a Canadian government-sponsored program on the harbor porpoise in the Bay of Fundy.

Liver preparations from a freshly killed Phocoena, analyzed immediately and after 24 hours storage at 4°C, had an oxygen uptake of 6445 and 6210 μL_2/gram/hour. Analyzed again after it was frozen at -20°C for 24 hours, the liver samples showed substantial, but reduced, uptake of oxygen (1053 µl O_2/gram/hour). Skin samples showed no detectable activity either fresh or after storage.

Tissues obtained from a Tursiops that died in captivity were analyzed after storage for 24 hours on ice. Liver consumed 1435 µL O_2/gram tissue/hour whereas epidermis consumed 92.4 µL O_2/gram tissue/hour, or approximately 7% of the activity of the liver.

Table 6.2:9

Species	Specimen (n)	No. of Tests	Reaction time (hrs)	Mean O_2 consumption ($\mu l/g$ tissue/hr)	Range	General Comments
truncatus Mont. Aq.	skin (4)	44	1	35.0	4.5-80.0	-variability may be due to: (1) lack of homogeneity in skin - sucrose suspension (2) poor condition and preservation of samples
25	skin (4)	8	2.5	61.4	49.0-73.8	
	skin (4)	4	3	27.2	-	
	skin (4)	11	4	21.9	5.8-31.8	
phocoena						
80-440-Pp	skin (4)	4	1.5	-	-	
	skin (4)	4	3.75	6.6	-	
breviceps						
80-394-Kb	skin (2)	2	1	38.3	0-83.3[a]	
	skin (2)	2	2	10.0	-	
	skin (2)	2	3	9.6	-	
	skin (2)	2	1	15.5	-	
bit						
ctolagus iculus BLM-1	liver (4) fresh	4	1	2715.0	2665-2805	-immediate loss of 50% on freezing and continued loss to 20% of original value after 14 days
	liver (4) frozen 1 day	4	1	1315.0	1285-1365	
	liver (4) frozen 2 days	4	1	895.0	755-1160	
	liver (3) frozen 7 days	3	1	785.0	770-800	
	liver (4) frozen 14 days	4	1	513.0	406-597	

sing equivalent preparations results were obtained for one of 3 trials. One failure was due to a fluctuation in the l; the cause of the other was unknown.

Subsequent studies on Tursiops, Kogia and Phocoena showed a wide range in the rate of oxygen consumption in cetacean skin, with substantially lower activity than was found in rabbit liver (Table 6.2:9). Tursiops and Kogia values were equivalent, in the range of 30-35 and 10-38 μL O_2/g tissue/hour respectively, compared with Phocoena, the values of which were 6.6 μL O_2/g tissue/hour.

These findings indicate that cetacean epidermal cells have low detectable metabolic activity as measured by the tissue respirometry technique. This can be taken as an indication that skin is less active than certain morphological and biochemical constituents would indicate, or that the technique was not appropriate for use in cetacean epidermal cell preparations.

Summary

This series of biochemical and physiological studies has improved our understanding of the nature and function of cetacean skin. It has confirmed the presence and helped to characterize the activity of certain enzymes and enzyme systems in epidermal cells, yet has failed to reveal the purpose of such activity. Our investigations on metabolic activity showed that the use of conventional techniques was not an appropriate approach to studying the physiology of cetacean skin. Of the systems tested, only the enzymes involved in phospholipid synthesis appeared to be affected by exposure to petroleum hydrocarbons. The implications of this effect on the epidermal cells and on the skin as a whole, are unknown at this time and require further study.

References

(1) Engelhardt, F.R. 1974. Aspects of vitamin E deprivation in the harp seal, Pagophilus groenlandicus. Unpublished. Ph.D. Thesis. p. 153.

(2) Folch, J., M. Lee and G.H. Sloan-Stanley. 1957. J. of Biol. Chem. 226: 497.

(3) Geraci, J.R. and D.J. St. Aubin. 1979. Tissue sources and diagnostic value of circulating enzymes in cetaceans. J. Fish. Res. Bd. Can. 36: 158-163.

(4) Herzfeld, A. and O. Greengard. 1971. Aspartate aminotransferase in rat tissue: changes with growth and hormones. Biochem. Biophys. Acta. 237: 88.

(5) Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with the folin phenol reagent. J. of Bio. Chem. 193: 265-275.

(6) Mercer, D.W. 1974. Separation of tissue and serum creatine kinase isoenzymes by ion-exchange column chromatograpy. Clin. Chem. 20: 36-40.

(7) St. Aubin, D.J. and J.R. Geraci. 1980. Tissue levels of ascorbic acid in marine mammals. Comp. Biochem. Physiol. 66A: 605-609.

(8) Umbreit, W.W., R.H. Burris and J.F. Stauffer. 1972. In Manometric and Biochemical Techniques. (Fifth Edition), Burgess Publishing Co., Minneapolis, MN 55415.

7.00 EFFECTS OF INHALATION OF PETROLEUM VAPORS

Petroleum vapors are harmful and can be fatal. This is particularly true of volatile sustances such as n-hexane, benzene, xylene, trimethyl-benzene, and up to and including C_{11}-C_{12} hydrocarbons (Wang and Irons 1961, Nau et al. 1966, Herskowitz et al. 1971, Carpenter et al. 1975b, 1978) concentrated in confined spaces. Hydrocarbons of C_{15} and greater are not harmful (Carpenter et al. 1976) except under laboratory conditions where unnaturally high vapor concentrations can be produced mechanically. The consequences of such exposures are documented for humans and numerous domestic and laboratory animal species. In this review, we draw upon these data to assess the hazards of exposure to cetaceans under natural oceanic conditions.

Eye irritation due to petroleum exposure in marine mammals has been reported in ringed seals (Geraci and Smith 1976) in which conjunctivits and corneal erosions were associated with direct contact with crude oil and its vapors. Three hours after the seals were placed in clean sea water, the condition began to resolve, and was no longer apparent after 20 hours. People report temporary eye irritation when exposed to gasoline vapors of 140 ppm for 8 hours (Drinker et al. 1943), and 500 ppm for 30 minutes to 1 hour (Drinker et al. 1943, Davis et al. 1960). Under conditions of occupational exposure, tanker and refinery workers showed no evidence of increased eye damage (Raitcheva and Zlatarev 1970, Anon. 1975, Theriault and Goulet 1979). Those exposed to jet fuel had a higher than normal incidence of chronic conjunctivitis, though the relationship is tenuous (Knave et al. 1978).

Serious eye damage can be produced by exposing animals to artifically high concentrations of petroleum vapors. Under such conditions, dogs

subjected to aerosol droplets of mixed xylenes at concentrations of 1250 ppm (Carpenter et al. 1975b), and rats to naphtha at 3400 ppm (Carpenter et al. 1975c), n-nonane at 880 ppm (Carpenter et al. 1978) and high aromatic solvent (HAS) at 1500 ppm (Carpenter et al. 1977a), developed irritation and inflammation of the mucus membranes of the eyes. Nau et al. (1966) produced cataracts in rats with prolonged exposure to over 600 ppm of C_9-C_{10}. However when rats were exposed to the highest concentration of HAS naturally attainable (66 ppm) they showed no evidence of eye irritation even after 390 hours over a 13 week period (Carpenter et al. 1977a).

Inhalation of highly concentrated petroleum vapors can lead to inflammation and damage of the mucous membranes of the airways, lung congestion, emphysema, pneumonia, hemorrhage, and death within minutes. For example, 5 minutes after entering an unpurged empty tank containing up to 16,000 ppm aviation gasoline vapors, a mechanic died with acute exudative tracheobronchitis (Wang and Irons 1961). Following an automobile accident, a boy exposed to gasoline vapors in excess of 10,000 ppm for 5 to 10 minutes died of manifest lung damage (Ainsworth 1960). Generally it is accepted that such exposure to gasoline vapor concentrations in excess of 10,000 ppm is rapidly fatal (Machle 1941).

Exposure to less concentrated vapors can also affect the respiratory system, but not as dramatically. Rats, guinea pigs, rabbits, monkeys, and dogs forced to breathe 230 ppm mineral spirits, (Rector et al. 1966), or 500 to 1000 ppm C_9-C_{10} hydrocarbons for up to 21 weeks (Nau et al. 1966) and people who habitually inhale gasoline vapors over periods of several years (Valpey et al. 1978) can develop inflammation, hemorrhage, and congestion of the lungs. Yet, inhalation of these lower concentrations of petroleum vapors is not necessarily associated with lung damage (Carpenter

et al. 1975, a,b,c, 1976, 1977 a and b, 1978).

Inhaled petroleum vapors are absorbed into the bloodstream and carried throughout the body. The effect on the central nervous system can be dramatic. Since the early 1960's there have been numerous reports of children and young adults intentionally sniffing gasoline (Lawton 1961, Oldham 1961, Grant 1962, Tolan and Lingl 1964, Runion 1975, Poklis and Burkett 1977, Boeckx, Postl and Coodin 1977, Valpey et al. 1978, Robinson 1978, Seshia et al. 1978, Kaufman and Wiese 1978, Hunter et al. 1979). Symptons closely resemble those of "model physcosis" (Toland and Lingl 1964), and include hallucinations, distortion of time and space, loss of appetite, drowsiness, altered shapes and colors and/or dizziness. Side effects are nausea (Wilson 1976, Knave et al. 1978), vomiting, uncoordination (Machle 1941), convulsions (Petri 1908), headache (Plummer 1913), coma (Machle 1941, Lawrence 1945), and death (Haggard 1920, Ainsworth 1960, Wang and Irons 1961).

Repeated inhalation of petroleum vapors over a long term results in degenerative changes in the brain (Valpey et al. 1978, Robinson 1978) and peripheral nerves (Machle 1941, Herskowitz et al. 1971, Knave et al. 1978). Not all of these consequences are due to the direct toxic effects of petroleum. Additives, principally tetraethyl lead, can accumulate in tissues, and cause damage that enhances the harmful influence of gasoline alone (Robinson 1978).

It is difficult to determine with certainty the concentrations of each vapor required to produce behavioral effects. The best data are available for gasoline. At room temperature, 15 to 18 deep forced inhalations of concentrated gasoline vapors are required to produce the "desired" effect (Poklis and Burkett 1977). Sniffing vapors at 10,000 ppm for 4 minutes results in dizziness (Poklis and Burkett 1977), and after 10 minutes, obvious intoxication (Runion 1975). Human volunteers breathing unleaded

gasoline vapors at 2600 ppm for 1 hour experienced dizziness and slight loss of coordination (Drinker et al. 1943). Vapor concentrations of 300 to 1000 ppm for weeks or months can produce dizziness in sensitive individuals (Machle 1941, Drinker et al. 1943, Herskowitz et al. 1971, Knave et al. 1978). Concentrations below this are generally innocuous (Lykke et al. 1979, Carpenter et al. 1975b, ACGIH 1971).

Prolonged exposure to concentrated petroleum vapors can affect other organs, including liver (Nau et al. 1966, Raitcheva and Zlatarev 1970), stomach (Machle 1941), kidneys (Nau et al. 1966), adrenals (Case 1972, Case and Coffman 1973), and the hematopoietic system (Nau et al. 1966, Rector et al. 1966 Prager and Peters 1970).

Effects on the latter are highly variable. Both the erythrocytic (red cell) and myelocytic (white cell) precursors can be stimulated, depressed, or remain unaffected. In humans, long term exposure to vapors has been associated with anemia (Edwards 1960, McLean 1960, Prager and Peters 1970). Straight-chain hydrocarbons such as n-decane have no effect on the marrow of rats whereas benzene or other aromatics can, but the effects are reversible within 2-6 months.

There is an equivocal relationship between maternal exposure to petroleum vapors and the health of the fetus. In pregnant rats, vapors of fuel oil or kerosene (400 ppm) or unleaded gasoline (1600 ppm) had no effect on fetal growth, development, or survival (Litton Bionetics, Inc. 1978, 1979 a,b). However pregnant women who intentionally sniff gasoline risk mental retardation of their offspring, particularly if the gasoline contains tetraethyl lead (Hunter et al. 1979).

In summarizing the effects of inhaling petroleum compounds, we integrated exposure time with the vapor concentations of some of the more harmful substances, in relation to known effects on humans and laboratory

animals (Figure 7.1). We have graded the effects into 4 categories according to their severity: death associated with destruction of lung and nervous tissue, disorders of the central nervous system including behavioral alterations, irritation to mucous membranes of the eyes and airways, and a category of exposure conditions within which no obvious effects have been noted. This safe limit has been defined for a number of compounds to which people are exposed on a day-to-day basis (Table 7.1). Generally, the aliphatic compounds are not as toxic as the aromatic substances of similar molecular weight. We accept these thresholds as applicable to a cetacean.

There are no reliable data on vapor concentrations of hydrocarbons associated with an oil spill. We therefore calculated these concentrations using partial pressure, temperature, and concentration of each constituent in a typical light crude oil. To do so, we assumed an improbably conservative set of circumstances in which all of the volatile substances in a 5 mm slick would evaporate instantaneously and completely into a 1 m layer of static air above the surface, resulting in exposure of an animal to an artifically maximized concentration of these substances. We used Murban oil as a representative light crude, and independently calculated the partial pressures and vapor concentration of each volatile component, based on its concentration in the oil (McAuliffe 1977). At 5° and 25°C, the combined vapor concentrations of the hydrocarbons considered would be 32 and 35 ppm, respectively (Table 7.2). For spills of more volatile substances, these concentrations could conceivably be much higher, however when we take into account such factors as diffusion, disperison of the slick, wind, and a more gradual release of the hydrocarbons (half life of 60-90 min. for most C_6 to C_{10} substances), it is unlikely that vapor concentrations can reach critical levels (Figure 7.1) for more than a few hours.

Figure 7.1

Summary of the effects of exposure to and inhalation of various petroleum vapors, principally those of gasoline. The duration of exposure and concentration of the vapors have been integrated to predict four levels of effects. Data are based on the reports listed below.

(1) Ainsworth 1980
(2) Davis et al. 1960
(3) Drinker et al. 1943
(4) Gamberale et al. 1975
(5) Haggard 1920
(6) Lykke and Stewart 1978
(7) Lykke et al. 1979
(8) Machle 1941
(9) Nau et al. 1966
(10) Poklis and Burkett 1977
(11) Registry of Toxic Effects of Chemical Substances, 1981
(12) Runion 1975
(13) Stewart et al. 1979
(14) Wang and Irons 1961

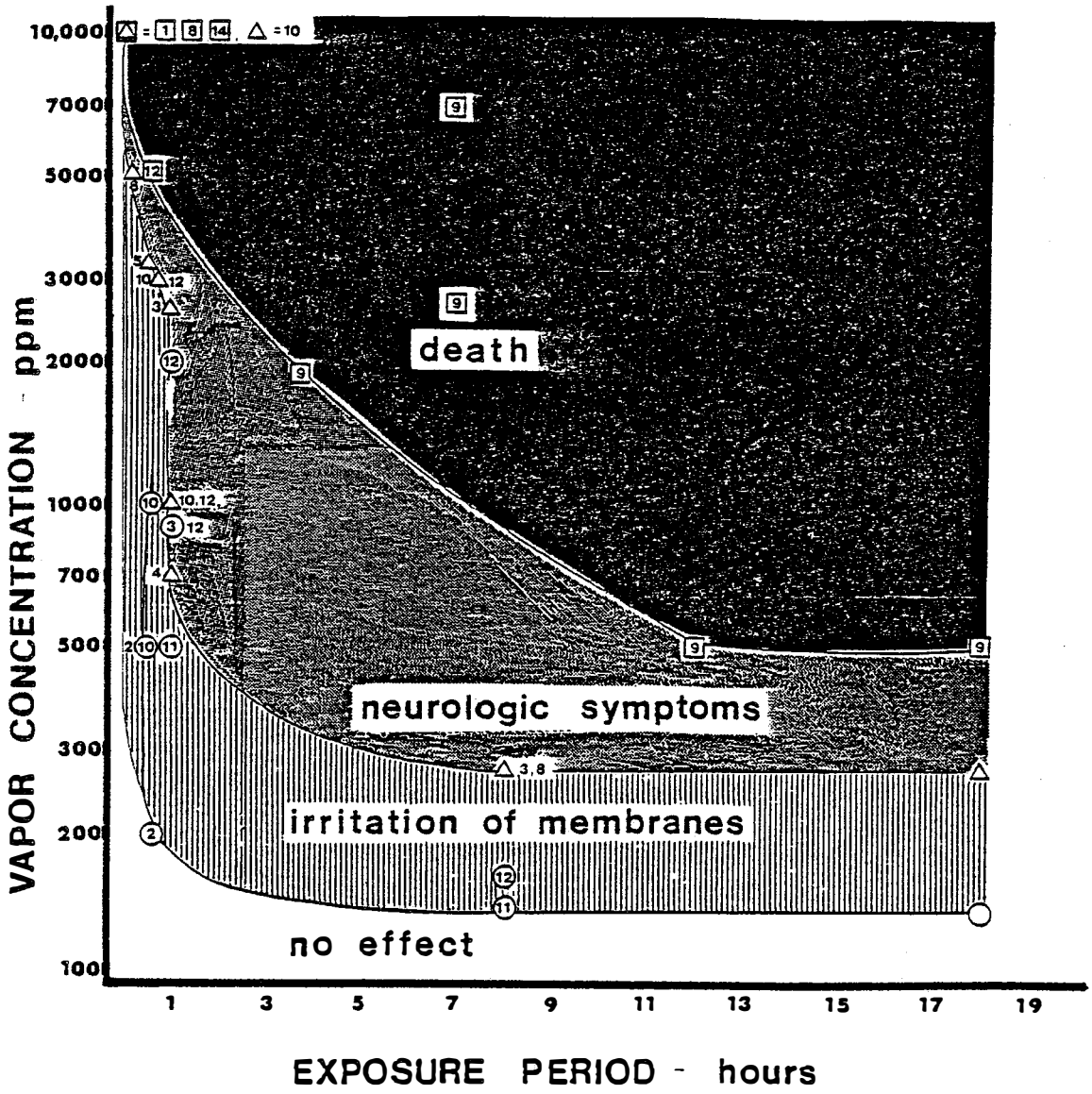
VAPOR CONCENTRATION - ppm
10,000
7000
5000
3000
2000
1000
700
500
300
200
100
death
neurologic symptoms
irritation of membranes
no effect
1
3
5
7
9
11
13
15
17
19
EXPOSURE PERIOD - hours

Figure 7.1

Table 7.1 Recommended safe limits for occupational exposure to various petroleum vapors[a].

Hydrocarbon	Recommended Safe Limit (ul/l = ppm)
n-pentane	500
n-hexane	500
cyclohexane	300
heptane	500
octane	400
benzene	10[b]
xylene	100
toluene	200[b]
ethylbenzene	100
trimethyl benzene	25
naphthalene	10
gasoline	300

[a] American Conference of Governmental Industrial Hygienists (1971).
[b] based on 8 hr. time-weighted exposure at 25°C, 1 atm pressure.

Table 7.2 Maximum theoretical concentration of petroleum hydrocarbons instantaneously released and confined in a 1 m layer of static air above a 5 mm thick slick of Murban crude oil.

Hydrocarbon	Formula	Concentration in Murban Crude (µg/l=ppb)[a]	Vapor Concentrations (ppm=µl/l) in air, at 5°C	25°C
aliphatic				
n-pentane	C_5H_{12}	1340	2.1	2.3
n-hexane	C_6H_{14}	1350	1.8	1.9
methylcyclopentane	C_6H_7	355	0.5	0.6
cyclohexane	C_6H_{12}	410	0.6	0.6
heptane	C_7H_{16}	330	0.4	0.4
octane	C_8H_{18}	1000[b]	1.0	1.1
nonane	C_9H_{20}	1000[b]	0.9	1.0
decane	$C_{10}H_{22}$	1000[b]	0.8	1.0
dodecane	$C_{12}H_{26}$	1000[b]	0.7	0.7
hexadecane	$C_{16}H_{34}$	1000[b]	0.5	0.5
naphthalene	$C_{10}H_8$	1000[b]	0.9	1.0
aromatic				
benzene	C_6H_6	6080	8.9	9.5
toluene	C_7H_8	6160	7.6	8.2
ethylbenzene	C_8H_{10}	825	0.9	1.0
total xylenes	C_8H_{10}	2950	3.2	3.5
trimethylbenzene	C_9H_{12}	750	0.7	0.8
diisopropylbenzene	$C_{12}H_{18}$	1000[b]	0.7	0.8

[a] from McAuliffe (1977)

[b] Not analyzed in Murban Crude. Concentrations arbitrarily established as upper limit.

If a whale or dolphin were unable to leave the immediate area of the source of the spill or were confined to a contaminated lead or bay, it would undoubtedly inhale some vapors, enough perhaps to cause some damage. The effect would depend more on the susceptibility of the animal, since the theoretically attainable concentrations of vapor are not high enough to pose a threat. Thus cetaceans that are stressed by lung and liver parasites, and adrenal disorders (Geraci and St. Aubin 1979) might be particularly vulnerable to the effects of even low levels of hydrocarbon vapors. Animals that are away from the immediate area, or exposed to weathered or residual oils would not be expected to suffer any consequences from inhalation, regardless of their condition.

References

(1) Ainsworth, R.W. 1980. Petrol vapour poisoning. Br. Med. J. 1: 1547-1548.

(2) American Conference of Governmental Industrial Hygienists (ACGIH). 1970. Threshold limit values of airborne contaminants for 1970. U.S. Fed. Register. Vol. 36 (105).

(3) Anon. 1975. Hazards of the oil industry. Br. Med. J. 3 (5983): 556.

(4) Boeckx, R.L., B. Postl and F.J. Coodin. 1977. Gasoline sniffing and tetraethyl lead poisoning in children. Pediatrics 60 (2): 140-145.

(5) Carpenter, C.P., E.R. Kinkead, D.L. Geary, Jr., L.J. Sullivan and J.M. King. 1975a. Petroleum hydrocarbon toxicity studies. IV. Animal and human response to vapors of rubber solvent. Toxicol. Appl. Pharmacol. 33 (3): 526-542.

(6) Carpenter, C.P., E.R. Kinkead, D.L. Geary, Jr., L.J. Sullivan and J.M. King. 1975b. Petroleum hydrocarbon toxicity studies. V. Animal and human response to vapors of mixed xylenes. Toxicol. Appl. Pharmacol. 33 (3): 543-548.

(7) Carpenter, C.P., E.R. Kinkead, D.L. Geary, Jr., L.J. Sullivan and J.M. King. 1975c. Petroleum hydrocarbon toxicity studies. II. Animal and human response to vapors of Varnish Makers' and Painters' naphtha. Toxicol. Appl. Pharmacol. 32: 263-281.

(8) Carpenter, C.P., D.L. Geary, Jr., R.C. Myers, D.J. Nachreiner, L.J. Sullivan and J.M. King. 1976. Petroleum hydrocarbon toxicity studies. XI. Animal and human response to vapors of deodorized kerosene. Toxicol. Appl. Pharmacol. 36: 443-456.

(9) Carpenter, C.P., D.L. Geary, Jr., R.C. Myers, D.J. Nachreiner, L.J. Sullivan and J.M. King. 1977a. Petroleum hydrocarbon toxicity studies. XIV. Animal and human response to vapors of high aromatic solvent. Toxicol. Appl. Pharmacol. 41 (2): 235-249.

(10) Carpenter, C.P., D.L. Geary, Jr., R.C. Myers, D.J. Nachreiner, L.J. Sullivan and J.M. King. 1977b. Petroleum hydrocarbon toxicity studies. XVI. Animal response to vapors of naphthenic aromatic solvent. Toxicol. Appl. Pharmacol. 41 (2): 261-270.

(11) Carpenter, C.P., D.L. Geary, Jr., R.C. Myers, D.J. Nachreiner, L.J. Sullivan and J.M. King. 1978. Petroleum hydrocarbon toxicity studies. XVII. Animal response to n-none vapor. Toxicol. Appl. Pharmacol. 44 (1): 53-61.

(12) Case, A.A. 1972. Toxicosis of public health interest: children and animals poisoned by a volatile agent in discarded motor oil. Clin. Toxicol. 5 (2): 267-270.

(13) Case, A.A. and J.R. Coffman. 1973. Waste oil toxic for horses. Vet. Clin. North Am. 3: 273-277.

(14) Davis, A., L.J. Schafer and Z.G. Bell. 1960. The effects on human volunteers of exposure to air containing gasoline vapors. Arch. Environ. Health 1: 548-554.

(15) Drinker, P., C.P. Yaglou and M.F. Warren. 1943. The threshold toxicity of gasoline vapor. J. Ind. Hyg. 25: 225-232.

(16) Edwards, R.V. 1960. A case report of gasoline sniffing. Am. J. Psych. 117: 555-557.

(17) Gamberale, F., G. Annwall and M. Hultengien. 1975. Exposure to white spirit. II. Psychological functions. Scand. J. Work Environ. Health 1: 31-39.

(18) Geraci, J.R. and D.J. St. Aubin. 1979. Stress and disease in marine mammals. In Biology of Marine Mammals: Insights through Strandings. NTIS Report PB-293890: 223-233.

(19) Grant, W.B. 1962. Inhalation of gasoline fumes by a child. Psychiatr. Q. 36: 555-557.

(20) Haggard, H.W. 1920. The anesthetic and convulsant effects of gasoline vapor. J. Pharmac. Exp. Therap. 16: 401-404.

(21) Herskowitz, A., N. Ishii and H. Schaumburg. 1971. N-hexane neuropathy: a syndrome occurring as a result of industrial exposure. N. Engl. J. Med. 285 (2): 82-85.

(22) Hunter, A.G.W., D. Thompson and J.A. Evans. 1979. Is there a fetal gasoline syndrome? Teratology 20 (1): 75-80.

(23) Kaufman, A. and W. Wiese. 1978. Gasoline sniffing leading to increased lead absorption in children. Clin. Pediatr. 17 (6): 475-477.

(24) Knave, B., B. Anshelm Olson, S. Elofsson, J. Gamberale, A. Isaksson, P. Mindus, H.E. Persson, G. Struwe, A. Wennberg and P. Westerholm. 1978. Long term exposure to jet fuel. II. A cross-sectional epidemiologic investigation on occupationally exposed industrial workers with a special reference to the nervous system. Scand. J. Work Environ. Health 4: 19-45.

(25) Lawton, J.L. and C.P. Malmquist. 1961. Gasoline addiction in children. Psychiatr. 35: 555-561.

(26) Litton Bionetics Inc. 1978. Teratology study in rats. Unleaded gasoline. Final report. API Med. Res. Publ. 2660014. 23 pp.

(27) Litton Bionetics Inc. 1979a. Teratology study in rats. Kerosene. Final report. API Med. Res. Publ. 2732175. 29 pp.

(28) Litton Bionetics Inc. 1979b. Inhalation/teratology study in rats. Fuel oil. Final report. API Med. Res. Publ. 2730483. 29 pp.

(29) Lykke, A.W.J. and B.W. Stewart. 1978. Fibrosing alveolitis (pulmonary interstitial fibrosis) evoked by experimental inhalation of gasoline vapours. Experientia 34 (4): 498.

(30) Lykke, A.W.J., B.W. Stewart, P.J. O'Connell and S.M. Le Mesurier. 1979. Pulmonary responses of atmospheric pollutants. I. An ultrastructural study of fibrosing alveolitis evoked by petrol vapor. Pathology 11 (1): 71-80.

(31) Machle, W. 1941. Gasoline intoxication. J. Am. Med. Assoc. 117 (23): 1965-1971.

(32) McAuliffe, C.D. 1977. Dispersal and alteration of oil discharged on a water surface. In Fate and Effects of Petroleum Hydrocarbons in Marine Organisms and Erosystems. (D.A. Wolfe, Ed.). Pergamon Press, Toronto. 478 pp.

(33) McLean, J.A. 1960. Blood dyscrasia after contact with petrol containing benzol. Med. J. Aust. 42: 845-849.

(34) Nau, C.A., J. Neal and M. Thornton. 1966. C_9-C_{12} fractions obtained from petroleum distillates. An evaluation of their potential toxicity. Arch. Environ. Health 12: 382-393.

(35) Oldham, W. 1961. Deliberate self-intoxication with petrol vapor. Br. Med. J. 5268: 1687-1688.

(36) Plummer, S.W. 1913. Case of petrol intoxication. Br. Med. J. 1: 661.

(37) Poklis, A. and C. Burkett. 1977. Gasoline sniffing: a review. Clin. Toxicol. 11: 35-41.

(38) Prager, D. and C. Peters. 1970. Development of aplastic anemia and the exposure to Stoddard solvent. Blood 35: 286-287.

(39) Raitcheva, V. and O. Zlatarev. 1970. Chronic effect of petroleum derivatives on enzyme constellations. Ind. Med. Surg. 39: 345-346.

(40) Rector, D.E., B.L. Steadman, R.A. Jones and J. Siegel. 1966. Effects on experimental animals of long-term inhalation exposure to mineral spirits. Toxicol. Appl. Pharmacol. 9: 257-268.

(41) Robinson, R.O. 1978. Tetraethyl lead poisoning from gasoline sniffing. J. Am. Med. Assoc. 240 (13): 1373-1374.

(42) Runion, H.E. 1975. Benzene in gasoline. Am. Ind. Hyg. Assoc. J. 36: 338-350.

(43) Seshia, S.S., K.R. Rajani, R.L. Boeckx and P.N. Chow. 1978. The neurological manifestations of chronic inhalation of leaded gasoline. Dev. Med. Child Neurol. 20 (3): 323-324.

(44) Stewart, B.W., S.M. Le Mesurier and A.W.J. Lykke. 1979. Correlation of biochemical and morphological changes induced by chemical injury to the lung. Chem.-Biol. Interact. 26 (3): 321-338.

(45) Theriault, G. and L. Goulet. 1979. A mortality study of oil refinery workers. J. Occup. Med. 21 (5): 367-370.

(46) Tolan, E.J. and F.A. Lingl. 1964. "Model psychosis" produced by inhalation of gasoline fumes. Am. J. Psychiatr. 120: 757-761.

(47) U.S. Dept. of Health, Education, and Welfare. National Institute for Occupational Safety and Health. 1981. Registry of toxic effects of chemical substances. Cincinnati, OH.

(48) Valpey, R., S.M. Sumi, M.K. Copass and G.J. Goble. 1978. Acute and chronic progressive encephalopathy due to gasoline sniffing. Neurology 28 (5): 507-510.

(49) Wang, C.C. and G.U. Irons. 1961. Acute gasoline intoxication. Arch. Environ. Health 2: 714-716.

(50) Wilson, F.W. 1976. Toxicology of petroleum naphtha distillate vapors. J. Occup. Med. 18 (12): pp. 821.

8:00 OIL INGESTION AND BIOACCUMULATION

8.1 Introduction and Literature Review

Marine mammals exposed to an oil spill might conceivably ingest oil directly, or feed on contaminated prey. There are few studies of the consequences of oil ingestion in marine mammals, and none establishing critical or lethal doses. Oil ingestion has been implicated as a cause of death in harbor seals, Phoca vitulina, (Duguy and Babin 1975), otters, Lutra lutra (Baker et al. 1981) and polar bears, Ursus maritimus (Anon. 1981), though other factors such as stress, hypothermia and starvation may have played a role. Up to 75 ml of crude oil was administered to harp, Phoca groenlandica, and ringed seals, Phoca hispida, with little or no effect (Geraci and Smith 1976). A bottlenose dolphin, Tursiops truncatus, given small doses (2.5-5 ml) of machine oil daily for over 3 months, showed no clinical signs of organ damage or intoxication (see Section 8.5)

We have not undertaken oil ingestion studies as part of the present investigation. We will attempt therefore to identify the possible effects on cetaceans by reviewing the literature on other species, and interpreting these data with a view toward certain anatomical, behavioral and physiological features unique to cetaceans.

The effects of ingested oil depend on the amount consumed, the composition or nature of the oil, and whether or not the ingested oil is regurgitated and aspirated. Consumption of crude oil, gasoline, kerosene or diesel fuel has proved fatal in cows, goats, rabbits, dogs, rats, mice, chickens and humans (Bliss 1898, Eaton 1943. Deichmann et al. 1944, Coale 1947, Gerarde 1959, 1964, Ashkenazi and Berman 1961, Narasimham and Ganla 1967, Toofanian et al. 1979). The quantities ingested ranged from as little as 55 ml, to over 20 L. In some instances, the animals died within

hours, while others survived for weeks or months. Ruminants generally succumb later, after developing bloat caused by the more volatile fractions in light oils and gasoline (Rowe et al. 1973). In general, small doses are acutely fatal if aspiration occurs, while considerably larger quantities can be tolerated if the oil remains in the gastrointestinal tract.

Petroleum hydrocarbons aspirated into the lungs cause an acute and devastating pneumonia, characterized by hemorrhage, exudative bronchitis, emphysema, abscessation and pseudocyst formation (Scott 1944, Richardson and Pratt-Thomas 1951, Gerarde 1959, 1964; Gross et al. 1964, Dragsted and Rodbro 1965). Some investigators suggested that these effects were caused by blood-borne hydrocarbons absorbed from the gastrointestinal tract (Deichmann et al. 1944). Subsequent studies attempted to resolve this apparent controversy using several approaches: comparing the effects of kerosene administered into the trachea, stomach, intestine, arterial or venous systems (Richardson and Pratt-Thomas 1951, Wolfsdorf and Kundig 1972, Wolfsdorf 1976), preventing aspiration by ligating or transecting the esophagus, inserting an endotracheal tube, or using test animals such as rabbits, which rarely vomit (Wolfe et al. 1970, Wolfsdorf and Kundig 1972, Richardson and Pratt-Thomas 1951, Lesser et al. 1943). Severe lung damage results from kerosene dosages in the range of 0.2 - 1.0 ml/kg given intratracheally or intravenously (Lesser et al. 1943, Richardson and Pratt-Thomas 1951, Gerarde 1959 and 1964; Gross et al. 1963 and 1964; Schwartz et al. 1965, Barter et al. 1966, Wolfsdorf 1976). By contrast, no gross pathology was observed in the lungs of dogs given kerosene in dosages up to 24 g/kg into the stomach, after the esophagus was transected (Wolfe et al. 1970). Wolfsdorf and Kundig (1972) administered kerosene at a rate of 45 ml/kg intragastrically to tracheostomized monkeys, and observed lung edema and patchy hemorrhage, but not the acute exudative type of pneumonia characteristically associated with intratracheal or intravenous injection

of much smaller dosages (Richardson and Pratt-Thomas 1951). Gerarde (1964) determined that the LD_{50} for kerosene was 140 times greater when administered orally (without aspiration) than when given intratracheally.

Petroleum hydrocarbons retained in the gastrointestinal tract can adversely affect the digestive system. Ingested fuel oils cause intestinal irritation and hemorrhage in rats given 5-25 ml/kg (Elars 1979 a,b; 1980 a-d). Mice, rabbits and dogs show thinning of the stomach wall and roughened gastric mucosa after consuming 33-90 ml/kg of kerosene (Narasimham and Ganla 1967). Crude oils and diesel fuel affect rumen motility in cows and sheep, and fermentation or evaporation of volatile fractions in the rumen produce bloat (Rowe et al. 1973, Ranger 1976). Hemorrhagic diarrhea, enteritis and colitis can also result from ingestion of these substances (Gibson and Linzell 1948, Bumstead 1949, Elars 1979 a,b; 1980 a-d).

Oil in the intestinal tract can be either absorbed into the blood-stream or excreted in the feces. Harp seal pups fed crude oil dosages of 1-3 ml/kg had oil-stained hind flippers within 1.5 hours after ingestion, suggesting rapid gastrointestinal clearance (Smith and Geraci 1975). The lower molecular weight fractions are more readily absorbed, and can be detected in the blood of seals ingesting dosages as low as 0.2 ml/kg/day for 5 days (Engelhardt et al. 1977). Once in the blood stream, these fractions can have a cytotoxic effect on various other organs or tissues.

An important function of the liver is to metabolize or detoxify substances absorbed from the gut, thereby protecting other organ systems. Severe or repeated insults from foreign substances generally result in liver degeneration or destruction, and infiltration of fibrous tissue (Thomson 1978). These changes have been observed following ingestion of unknown quantities of diesel oil by goats (Toofanian et al. 1979), 3-18 ml/kg of

kerosene by rats (Ashkenazi and Berman 1961) 33-70 ml/kg of kerosene by mice, rabbits and dogs (Narasimham and Ganla 1967), and unknown quantities of gasoline by cows (Albert and Ramey 1964). In each case, the concentration of hydrocarbons in the blood was not determined, and it is therefore difficult to establish critical levels which will injure the liver. One indication is that kerosene at a dosage of 0.3 ml/kg injected directly into the portal vein of baboons, produces only mild liver damage (Wolfsdorf 1976).

Low doses can be detoxified by the liver without affecting its integrity. Seals ingesting 1-3 ml/kg of crude oil had no gross, microscopic or biochemical evidence of liver damage (Geraci and Smith 1976). Serum enzymes, used as sensitive indicators of hepatocellular destruction, were unchanged in a ewe consuming an unknown quantity of diesel oil even though the animal had other signs of liver dysfunction (hypoglycemia and ketosis) (Ranger 1976). In general, the liver can be affected if sufficient oil is absorbed into the bloodstream, but only rarely has death been attributable to liver failure following oil ingestion (Narasimham and Ganla 1967).

Animals consuming various petroleum compounds sometimes behave abnormally, suggesting damage to the central nervous system. The symptoms range from depression and lethargy to ataxia, convulsions, hypersensitivity and frenzy (Eaton 1943, Gibson and Linzell 1948, Bumstead 1949, Rowe et al. 1973, Gardner 1977, Toofanian et al. 1979). However, attempts to identify specific lesions in the brain have been unsuccessful (Narasimham and Ganla 1967, Wolfsdorf 1976). Only Luick (1977) described a cerebral abscess in a reindeer, 60 days after it had ingested 300 ml of Prudhoe Bay crude oil. The neurological symptoms may therefore be related to anoxia associated with aspiration pneumonia (Baldachin and Melmed 1964, Cachia and Fenech 1964, Wolfsdorf 1976) or to hypoglycemia following liver failure (Narasimham and

Ganla 1967).

Petroleum hydrocarbons are removed from the bloodstream by a variety of mechanisms, including excretion via the kidneys. Oil can be detected in the urine of rats ingesting 5-25 ml/kg of various fuel oils (Elars 1979 a,b; 1980 a-d), and of seals inhaling vapors for 24 hours while immersed in an oil slick (Engelhardt et al. 1977). Kerosene doses of 18-70 ml/kg in rats, mice, rabbits and dogs cause swelling and cloudy degeneraton of the kidney tubular epithelium, and congestion of the glomeruli (Ashkenazi and Berman 1961, Narasimham and Ganla 1967). More dramatic lesions such as necrosis and sloughing of the tubular epithelium were noted in a case of fatal diesel oil poisoning in goats (Toofanian et al. 1979).

A variety of other systemic effects have been inconsistently reported in experimental studies and case reports on oil ingestion. These include myocardial and pericardial hemorrhage (Narasimham and Ganla 1967, Meadows and Waltner-Toews 1979), skin irritation, hair loss and subcutaneous hemorrhage (Gardner 1977, Elars 1979 a,b; 1980 a-d), blindness (Eaton 1943, Rowe et al. 1973), periocular and conjunctival hemorrhage or congestion (Ranger 1976, Elars 1979 a,b; 1980 a-d), and peritonitis (Rowe et al. 1973). By contrast, Narasimham and Ganla (1967) found no heart or spleen abnormalities in mice, rabbits and dogs ingesting kerosene at a dosage of 33-70 ml/kg. Sheep tolerated up to 1100 g of Bunker C per day, without any outward signs of distress (MacIntyre 1970), and pigs drinking gasoline contaminated water (60 g/l) for 7 days were similarly unaffected (Mitchell et al. 1978).

Critical doses (LD_{50})for ingestion of fuel oils were established for rats in a series of studies (Elars 1979 a,b; 1980 a-d). For three types of No. 2 fuel oil, the LD_{50} ranged between 14.5 and 21.2 ml/kg. The toxicity of heavier fuel oils (No. 6) was more variable, ranging from

5.1 ml/kg for one type to over 25 ml/kg for the other two substances tested. Using these dosages as guidelines, we can calculate the amount of fuel oil that cetaceans would have to consume to be at risk (Table 8.1). With the exception of the smaller species or immature animals of all species, the quantities are well beyond the limits of what might be accidentally consumed by a cetacean at sea. Cetaceans do not drink sea water, and it is unlikely that they would ingest such quantities of crude oil.

Table 8.1: Estimate of quantities of fuel oils which cetaceans would have to ingest to pose a threat to survival.

Species	Average Adult Body Wt. (kg)	Volume of Fuel Oil[a] mean[b]	range[c]
Phocoena phocoena	70	1.05	.35-1.75
Tursiops truncatus	250	3.0	1.0-5.0
Globicephala sp.	800	30	10-50
Orcinus orca	3,000	75	25-125
Balaenoptera physalus	40,000	600	200-1,000

[a] volume in liters

[b] based on 15 ml/kg

[c] based on 5-25 ml/kg

The most serious threat to people consuming sublethal doses of petroleum hydrocarbons is pneumonia resulting from aspiration of vomitus. For this reason, gastric lavage is carried out cautiously (Podolsky 1952, Cachia and Fenech 1964). Cetaceans are uniquely protected from this complication by an anatomical adaptation which reduces or eliminates the possibility of aspirating regurgitated material. The laryngeal apparatus of these animals emerges from the ventral aspect of the pharynx and inserts into the nasal passages in the skull (Hosokawa 1950), allowing them to breathe and swallow simultaneously without the risk of choking. Similarly, regurgitated stomach contents would be blocked from entering the airways. The possibility of cetaceans developing aspiration pneumonia following ingestion of oil is therefore highly unlikely.

Oil ingestion may still pose a threat to cetaceans. Baleen plates might become fouled, thereby impairing a mysticete's ability to feed (see Section 9.0, this report). A second cause for concern is the long-term effect of ingesting food contaminated with petroleum hydrocarbon residues. Marine invertebrates and fish can accumulate certain fractions, particularly naphthalenes and tetramethylbenzene (McCain et al. 1978, Roesijadi et al. 1978), and these are transferred in turn to cetaceans, many of which are high level predators. Some petroleum hydrocarbons, such as benzopyrene, are potent carcinogens in a wide variety of invertebrate and vertebrate species (Hodgins et al. 1977), and would undoubtedly have a similar effect on cetaceans. The impact of the more commonly occurring fractions is more difficult to predict, and can only be addressed through careful monitoring of cetacean populations (see Section 8.2, this report). In an historical analysis of cetacean strandings on the coast of the Netherlands, van Bree (1977) associated two periods of sudden decline in the number of strandings

with an increase in pollutant levels in the North Sea. He reported that the first, which took place around 1946, was possibly linked to the dumping of war chemicals or the increase in the use of oil; the second, more recently, was "clearly related to the increase in pollution of the North Sea". If these associations are accurate, they suggest that pollutants, including petroleum hydrocarbons, can affect reproductive success. Organochlorine (PCB, DDT) residues have been associated with reproductive disturbances in seals (Helle et al. 1976), sea lions (Gilmartin et al. 1976), and birds (King et al. 1970, Blus et al. 1974). Hydrocarbon residues can affect reproductive success in birds (Holmes et al. 1968, Grau et al. 1977), but such correlations have not yet been demonstrated for mammals. Only recently have attempts been made to monitor these compounds in marine mammals (Section 8.2), and continued surveillance is necessary to establish if increased levels of these substances are associated with any functional or behavioral disturbances.

References

(1) Albert, T.F. and D.B. Ramey. 1964. Abomasal displacement associated with ingestion of gasoline. J. Am. Vet. Med. Assoc. 145: 460-461.

(2) Anon. 1981. Effects of crude oil on polar bears. Summary Report. Ministry of Indian and Northern Affairs, Canada. Report No. QS-8283-010-EE-A1. Ottawa. 17 pp.

(3) Ashkenazi, A.E. and S.E. Berman. 1961. Experimental kerosene poisoning in rats. Pediatrics 28: 642-649.

(4) Baker, J.R., A.M. Jones, T.P. Jones and H.C. Watson. 1981. Otter Lutra lutra L. mortality and marine oil pollution. Biol. Conserv. 20: 311-321.

(5) Baldachin, B.J. and R.N. Melmed. 1964. Clinical and therapeutic aspects of kerosene poisoning: a series of 200 cases. Br. Med. J. 2: 28-30.

(6) Barter, R.A., M.J. Byrne and R.F. Carter. 1966. Pulmonary hyaline membrane: late results of injury to the lung lining. Arch. Dis. Child. 41: 489-495.

(7) Bliss, E.C. 1898. Kerosene ingestion by a child. Iowa Health Bull. 13: 4, 1899.

(8) Blus, L.J., B.S. Neely, Jr., A.A. Belisle and R.M. Prouty. 1974. Organochlorine residues in brown pelican eggs: relation to reproductive success. Environ. Pollut. 7 (2): 81-91.

(9) Bumstead, W.A. 1949. Unusual case of crude oil poisoning of cattle. North Am. Vet. 30: 712.

(10) Cachia, E.A. and E.F. Fenech. 1964. Kerosene poisoning in children. Arch. Dis. Child. 39: 502-504.

(11) Coale, A.J. 1947. Drinking of crude oil by cattle. North Am. Vet. 28: 221.

(12) Deichmann, W.B., K.V. Kitzmiller, S. Witherup and R. Johansmann. 1944. Kerosene intoxication. Ann. Int. Med. 21: 803-823.

(13) Dragsted, P.J. and P. Rodbro. 1965. Pseudocysts of the lungs in kerosene poisoning: report of two cases. Dis. Chest 48: 87-90.

(14) Duguy, R. and P. Babin. 1975. Acute intoxication by hydrocarbons observed in a harbor seal (Phoca vitulina). Conseil International pour L'Exploration de la Mer, Comite de Mammiferes Marins, CM. 1975/N:5.

(15) Eaton, G. 1943. Paraffin poisoning in cattle. Vet. Rec. 55 (2): 19 pp.

(16) Elars Bioresearch Laboratories, Inc. 1979a. Acute toxicity tests of API178-3 No. 2 home heating oil (10% Cat). Project No. 1443. API Med. Res. Publ. 2732773, 52 pp.

(17) Elars Bioresearch Laboratories, Inc. 1979b. Acute toxicity tests of API78-4 No. 2 home heating oil (50% Cat). Project No. 1443. API Med. Res. Publ.

(18) Elars Bioresearch Laboratories, Inc. 1980a. Acute toxicity tests of API78-2 No. 2 home heating oil (30% Cat). Project No. 1443. API Med. Res. Publ. 2732771. 48 pp.

(19) Elars Bioresearch Laboratories, Inc. 1980b. Acute toxicity tests of API78-7 No. 6 heavy fuel oil (API gravity 17.1/0.8% S) Project No. 1443. API Med. Res. Publ. 2732774. 45 pp.

(20) Elars Bioresearch Laboratories, Inc. 1980c. Acute toxicity tests of API79-2 No. 6 heavy fuel oil (API gravity 5.2/1.2% S). Project No. 1443. API Med. Res. Publ. 2732813. 52 pp.

(21) Elars Bioresearch Laboratories, Inc. 1980d. Acute toxicity tests of API78-6 No. 6 heavy fuel oil (API gravity 11.7/2.7% S). Project No. 1443. API Med. Res. Publ. 2732814. 47 pp.

(22) Engelhardt, F.R., J.R. Geraci and T.G. Smith. 1977. Uptake and clearance of petroleum hydrocarbons in the ringed seal, Phoca hispida. J. Fish. Res. Bd. Can. 34: 1143-1147.

(23) Gardner, D.L. 1977. Toxicity of waste petroleum products in cattle. Vet. Med. Small Anim. Clin. 72 (12): 1874-1876.

(24) Geraci, J.R. and T.G. Smith. 1976. Direct and indirect effects of oil on ringed seals (Phoca hispida) of the Beaufort Sea. J. Fish. Res. Bd. Can. 33: 1976-1984.

(25) Gerarde, H.W. 1959. Toxicological studies on hydrocarbons. V. Kerosene. Toxicol. Appl. Pharmacol. 1: 462-474.

(26) Gerarde, H.W. 1964. Kerosene - experimental and clinical toxicology. Occup. Health Rev. 16: 17-21.

(27) Gibson, E.A. and J.L. Linzell. 1948. Diesel oil poisoning in cattle. Vet. Rec. 60: 60-61.

(28) Gilmartin, W.G., R.L. Delong, A.W. Smith, J.C. Sweeney, B.W. De Lappe, R.W. Risebrough, L.A. Griner, M.D. Dailey and D.B. Peakall. 1976. Premature parturition in the California sea lion. J. Wildl. Dis. 12: 104-115.

(29) Grau, C.R., T. Roudybush, J. Dobbs and J. Wathen. 1977. Altered yolk structure and reduced hatchability of eggs from birds fed single doses of petroleum oils. Science 195: 779-781.

(30) Gross, P., J.M. McNerney and M.A. Babyak. 1963. Kerosene pneumonitis: an experiment with small doses. Am. Rev. Respir. Dis. 88: 656-653.

(31) Gross, P., J.M. McNerney and M.A. Babyak. 1964. Kerosene pneumonitis: an experimental study with small doses. Tuberculology 21: 86-93.

(32) Helle, E., M. Olsson and S. Jensen. 1976. PCB levels correlated with pathological changes in seal uteri. AMBIO 5: 261-263.

(33) Hodgins, H.O., B.B. McCain and J.W. Hawkes. 1977. Marine fish and invertebrate diseases, host disease resistance, and pathological effects of petroleum. In Effects of Petroleum on Arctic and Subarctic Marine Environments and Organisms. Volume II. Biological Effects. Academic Press, N.Y. pp. 95-173.

(34) Holmes, W.N., J. Gorsline and J. Cronshaw. 1979. Effects of mild cold stress on the survival of sea water-adapted mallard ducks (Anas platyrhynchos) maintained on food contaminated with petroleum. Environ. Res. 20 (2): 524-544.

(35) Hosokawa, H. 1950. On the cetacean larynx, with special remarks on the laryngeal sack of the sei whale and the arytenoepiglottideal tube of the sperm whale. Sci. Rep. Whales Res. Inst., No. 3. pp. 23-62.

(36) King, K.A., E.L. Flickinger and H.H. Hildebrand. 1978. Shell thinning and pesticide residues in Texas aquatic bird eggs, 1970. Pestic. Monit. J. 12 (1): 16-21.

(37) Lesser, L.I., H.S. Weens and J.D. McKey. 1943. Pulmonary manifestations following the ingestion of kerosene. J. Pediatr. 23: 352-364.

(38) Luick, J.R. 1977. Industrial development in Alaska and its effects on the nutritional and physiological status of animals. Tech. Prog. Rep., Inst. of Arctic Biol., University of Alaska, Fairbanks, AL. RLO-2229-T3-150.

(39) MacIntyre, T.M. 1970. Effect of Bunker "C" oil on sheep. Can. J. Anim. Sci. 59: 748-749.

(40) McCain, B.B., H.O. Hodgins, W.D. Gronlund, J.W. Hawkes, D.W. Brown, M.S. Myers and J.H. Vandermeulen. 1978. Bioavailability of crude oil from experimentally oiled sediments to English sole, Parophrys vetulus and pathological consequences. J. Fish Res. Bd. Can. 35:

(41) Meadows, D.L. and D. Waltner-Toews. 1979. Toxicosis in dairy cattle: was it crude-oil poisoning. Vet. Med. Small Anim. Clin. 74 (4): 545-546.

(42) Mitchell, L.H., R.G. Brown and J.W. Dent. 1978. The effect of gasoline contamination of the water supply on the growth and water consumption of pigs. Can. Vet. J. 19 (1): 10-12.

(43) Narasimhan, M.J. Jr. and V.G. Ganla. 1967. Experimental studies on kerosene poisoning. Acta Pharmacol. (Kobenh) 25: 214-224.

(44) Podolsky, M.L. 1952. Pneumonia associated with the ingestion of petroleum products by children. Dis. Chest 21: 327-333.

(45) Ranger, S.F. 1976. A case of diesel oil poisoning in a ewe. Vet. Rec. 99 (25-26): 508-509.

(46) Richardson, J.A. and H.R. Pratt-Thomas. 1951. Toxic effects of varying doses of kerosene administration by different routes. Am. J. Med. Sci. 221: 531-536.

(47) Roesijadi, G., J.W. Anderson and J.W. Blaylock. 1978. Uptake of hydrocarbons from marine sediments contaminated with Prudhoe Bay crude oil: influence of feeding type of test species and availability of polycyclic aromatic hydrocarbons. J. Fish. Res. Bd. Can. 35: 608-614.

(48) Rowe, L.D., J.W. Dollahite and B.J. Camp. 1973. Toxicity of two crude oils and of kerosene to cattle. J. Am. Vet. Med. Assoc. 162: 61-66.

(49) Schwartz, S.I., R.C. Breslau, F. Kutner and D. Smith. 1965. Effects of drugs and hyperbaric oxygen environment on experimental kerosene pneumonitis. Dis. Chest 47: 353-359.

(50) Scott, E.P. 1944. Pneumonia, pneumothorax and emphysema following the ingestion of kerosene. J. Pediatr. 25: 31-34.

(51) Smith, T.G. and J.R. Geraci. 1975. The effect of contact and ingestion of crude oil on ringed seals of the Beaufort Sea. Beaufort Sea Tech. Rep. No. 5. 67 pp.

(52) Thomson, R.G. 1978. General Veterinary Pathology. W.B. Saunders Co., Toronto. 444 pp.

(53) Toofanian, F., S. Aliakbar and B. Ivoghi. 1979. Acute diesel fuel poisoning in goats. Trop. Anim. Health Prod. 11 (2): 98-101.

(54) Van Bree, P.J.H. 1977. On former and recent strandings of cetaceans on the coast of the Netherlands. Z. Saeugetierkd 42: 101-107.

(55) Wolfe, B.M., A.E. Brodeur and J.B. Shields. 1970. The role of gastrointestinal absorption of kerosene in producing pneumonitis in dogs. J. Pediat. 76 (6): 867-873.

(56) Wolfsdorf, J. 1976. Kerosene intoxication: an experimental approach to the etiology of the CNS manifestations in primates. J. Pediatr. 88 (6): 1037-1040.

(57) Wolfsdorf, J. and H. Kundig. 1972. Kerosene poisoning in primates. S. Afr. Med. J. 46: 619-621.

8.2 Petroleum Residues in Marine Mammal Tissues

Introduction

Ingested oil is usually detoxified and metabolized before it is excreted. Studies on fish indicate that some oil fractions, such as naphthalene and tetramethylbenzene, may persist in tissues after all of the other components have been cleared. To determine the extent that free ranging marine mammals may have accumulated petroleum fractions through ingestion of oil or oil-contaminated prey, we analyzed tissue samples for naphthalenes.

Materials and Methods

Analyses are being performed according to the method of Neff and Anderson (1975). Weighed tissue samples are homogenized in HPLC grade hexane using a Polytron homogenizer, centrifuged at 5,900 x g for 10 min., and the supernatants treated with florisil to remove interfering substances. After 24 hr., the samples are filtered through glass wool, and the absorbance values at 220 nm and 230 nm determined using a Perkin-Elmer spectrophotometer with deuterium lamp and quartz cuvette.

In our laboratory, naphthalene has an absorbance peak at 220 nm. Interfering substances in the tissues, which are not removed by the florisil, contribute to background absorbance and are measured at 230 nm. The absorbance (A) due to naphthalenes is calculated as the difference between the two readings (i.e. A220-A230).

The Absorbance Coefficient (E) for naphthalene, determined for our instrument from calibration data and standard curves, is 1.0558×10^5 mol^{-1} L cm^{-1}. Unknown concentrations (C), in mol L^{-1}, are calculated from the following equation:

$$C = \frac{A220 - A230}{E\ell}$$

where A = absorbance at 220 nm and 230 nm

E = absorbance coefficient

ℓ = path length = 1 cm

The reported values, expressed in ppm, are calculated as follows:

$$C \text{ (in ppm)} = \frac{A220 - A230}{1.0558 \times 10^{5}\ \text{mol}^{-1}\text{Lcm}^{-1} \times 1\text{cm}} \times 10^{-3}\text{Lx50mlx128g} \times \frac{10^{6}\ \text{ppm}}{\text{sample wt (g)}}$$

$$= \frac{A220 - A230}{\text{sample weight (g)}} \times 60.707$$

Results and Discussion

We analyzed 258 tissue samples from 13 marine mammal species (Table 8.2:1). Highest concentrations of naphthalene were present in the blubber of all species examined. Confidence limits of these data, based on internal standards and repeated analyses are ± 25%. The principal source of the variation is the adsorption of naphthalene onto florisil during the sample purification step. When known amounts of naphthalene are added to tissue homogenates, recovery ranges from 24 to 59% of the expected levels (Table 8.2:2). Therefore, the actual concentrations of naphthalene in marine mammal tissues may be up to 4 times higher than are reported here.

Two harbor seals suspected of having come into contact with crude oil had high concentrations of naphthalenes on their skin. Naphthalene levels in liver and blubber were not remarkable, indicating that the seals ingested little or no oil, or that ingested oil was rapidly metabolized. However, the presence of naphthalenes in brain, lung and kidney suggests that the seals may have assimilated oil by inhalation, or that oil residues are more

Table 8.2:1 Levels of naphthalene (in ppm) in tissues collected from stranded marine mammals

Species	Tissue	n	$\bar{x}$	SD	Range min	Range max
P. phocoena	liver	14	.95	1.69	0.1	6.6
	blubber	24	7.18	2.46	3.5	13.5
S. coeruleoalba	liver	4	.59	—	.08	1.9
	blubber	5	7.64	—	4.7	10.6
D. delphis	liver	4	.36	—	.09	1.0
	blubber	4	7.7	—	5.7	12.0
	heart	1	.6	—	—	—
	kidney	1	.07	—	—	—
	lung	1	.02	—	—	—
G. macrorhynchus	blubber	12	5.23	1.73	2.2	8.2
B. physalus	muscle	1	4.0	—	—	—
L. acutus	liver	42	.09	.17	.07	.89
	blubber	55	7.57	4.59	.03	25.1
G. melaena	liver	6	.25	.17	.08	.50
	blubber	31	3.91	3.46	.78	16.7
	kidney	5	.30	—	.00	.72
	lung	3	.07	—	.02	.13
	mammary	1	.00	—	—	—
	muscle	3	.49	—	.40	1.3
B. acutorostrata	liver	1	.3	—	—	—
	blubber	1	6.1	—	—	—
K. breviceps	liver	1	.06	—	—	—
	blubber	1	8.9	—	—	—
	kidney	1	.88	—	—	—
	heart	1	.13	—	—	—
	muscle	1	1.84	—	—	—
P. vitulina	liver	25	.11	.06	.03	.30
	blubber	19	4.77	2.89	2.00	11.0
	kidney	2	.22	—	.20	.23
	lung	1	.1	—	—	—
	intestine	2	.08	—	.05	1.0
	brain	1	.17	—	—	—
	muscle	2	.05	—	—	—
	skin	3	4.80	—	2.4	6.8
H. grypus	blubber	1	3.0	—	—	—
M. angustirostris	liver	1	.7	—	—	—
T. manatus	muscle	1	.2	—	—	—

Table 8.2:2 Recovery of naphthalene from internal standards

Tissue	Endogenous*	Naphthalene Added*	Expected*	Recovered*	% Recovery
Liver	< 0.07	2.81	< 2.88	< .68	24
Fat	< 1.0	32.6	< 33.6	< 19.4	59
Brain	< 1.0	28.5	< 29.5	< 16.3	55

* Concentrations in ppm naphthalene

persistent in these tissues.

The significance of the observed naphthalene levels in marine mammal tissues is difficult to assess. Fish exposed to oil spills or oil-water dispersions may concentrate up to 300 ppm of naphthalene in liver and bile, but these levels quickly decrease to less than 3 ppm within 2-3 days of depuration (Lee et al. 1972, Melancon and Lech 1978). There are no data available for "unexposed" fish taken at sea. In oil-exposed shrimp, the whole body concentration of naphthalene is less than 0.8 ppm after 24 hours exposure; these levels also fall rapidly to less than 0.2 ppm over a 1-4 day depuration period (Anderson et al. 1975, Sanborn and Malins 1977, Tatem 1977). Nevertheless, it appears that low level naphthalene residues in prey species accumulate in marine mammal tissues, particularly blubber. Mobilization of fat stores, such as might occur during migration or lactation, would produce transiently higher blood levels of hydrocarbons, while simultaneously making them more available for detoxification and excretion.

References

(1) Anderson, J.W. 1975. Laboratory studies on the effects of oil on marine organisms: an overview. API Publn. No. 4249. 70 pp.

(2) Lee, R.F., R. Sauerheber and G.H. Dobbs. 1972. Uptake, metabolism and discharge of polycyclic aromatic hydrocarbons by marine fish. Mar. Biol. 17: 201-208.

(3) Melancon, M .J., Jr. aand J.J. Lech. 1978. Distribution and elimination of naphthalene and 2-methylnaphthalene in Rainbow trout during short- and long-term exposures. Arch. Environ. Contam. Toxicol. 7: 207-220.

(4) Neff, J.M. and J.W. Anderson. 1975. An ultraviolet spectrophotometric method for the determination of naphthalene and alkylnaphthalenes in the tissues of oil-contaminated marine animals. Bull. Environ. Contam. Toxicol. 14: 122-128.

(5) Sanborn, H.R. and D.C. Malins. 1977. Toxicity and metabolism of naphthalene: a study with marine larval invertebrates. Proc. Soc. Exper. Biol. Med. 154: 151-155.

(6) Tatem, H.E. 1977. Accumulation of naphthalenes by grass shrimp: effects on respiration, hatching and larval growth. In: Fate and Effects of Petroleum Hydrocarbons in Marine Ecosystems and Organisms (D.A. Wolfe, Ed.). Pergamon Press, Toronto. pp. 201-209.

8.3 Metabolism of Ingested Oil - Studies on Cytochrome P450 in Cetaceans

Introduction

Cytochrome P450 is an iron-containing protein found in liver cells. It is part of a dynamic enzyme system that is involved in the metabolism and detoxification of a wide variety of foreign componds, including components of crude oil. By comparing liver cytochrome P450 levels in apparently healthy dolphins with those which are suspected to have come into contact with an oil spill, we may be able to ascertain whether the exposed animals had ingested and were metabolizing oil. Such data would necessarily be supported by tissue analyses for residues of petroleum and other pollutants.

Materials and Methods

Fresh liver samples were collected from a juvenile Tursiops truncatus which died accidentally during a live capture operation in the Gulf of Mexico (cf. NMFS permit #287), from a 1-year-old Tursiops (MH-79-180-Tt) which died in captivity at the New England Aquarium, Boston, MA, from 3 Atlantic white-sided dolphins which stranded alive along the New England coast and from a harbor porpoise, Phocoena phocoena, taken in Canadian waters as part of an ongoing research program sponsored by the Canadian government. The tissues were removed within one-half hour of death, placed on ice, and transported to Guelph, where they were analyzed within 6-24 hours of sampling. Liver samples from laboratory animals were obtained for comparative data, and to assess the stability of the cytochrome system under various storage conditions.

Liver microsomes were isolated and analyzed for total heme, cytochrome P450 and cytochrome b_5 (Omura and Sato 1964). Homogenized liver was centrifuged at 12,000 x g for 25 min. at 4°C, and the resultant supernatant was then centrifuged at 78,000 x g for 90 min. at 4°C to sediment

the microsomes. The pellet of microsomes was resuspended in cold isotonic (1.15%) KCl. The homogenization, high speed centrifugation, and resuspension were then repeated.

Protoheme content was determined after converting the heme into pyridine-hemochromogen in the presence of 0.1N NaOH and 20% pyridine. The absorption peak at 556 nm was quantified using a molar extinction coefficient of 32.4 cm^{-1} mM^{-1}, and the difference in spectra between the dithionite-reduced and air-oxidized hemochromogen.

Cytochrome b_5 was determined from the difference in spectra between NADH-reduced and the air-oxidized microsomes. This yielded an absorption peak at 425 nm with an increment of molar extinction of 185 cm^{-1} mM^{-1}.

For the determination of cytochrome P450, the sample was first saturated with carbon monoxide, then reduced with a few milligrams of solid sodium dithionite. The broad peak at 450 nm was quantified using an increment of molar extinction of 91 cm^{-1} mM^{-1}.

Microsomes were analyzed for iron after digestion in sulfuric acid and neutralization with NaOH. The iron was quantified spectrophotometrically using 1.10 phenanthroline (Schilt 1969). Protein was analyzed by the method of Lowry et al. (1951), using human serum albumin as the standard. All specrophotometric measurements were carried out using a Unicam SP1800[1] ultra-violet double beam spectrophotometer.

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[1] Pye Unicam Corp., England

Results and Discussion

Livers of the cetaceans examined contained cytochrome P450; cytochrome b_5 and total heme, generally in somewhat lower concentrations than are found in rats and rabbits (Table 8.3:1). One white-sided dolphin had considerably higher direct comparisons, however, may not be appropriated due to gross differences in body size and liver weight. The concentration of P450 in cetaceans is closer to that found in the baboon (Autrup et al. 1975). One white-sided dolphin, had considerably higher levels of P450, which may either reflect induction or individual variation.

The ability of cetaceans to detoxify ingested oil would depend in part on induction of the cytochrome system. A pilot study on oil ingestion in rats (Section 8.4) has shown that oil is a potent inducer of P450, and if dolphins are similarly exposed to crude oil, we expect to observe comparable changes in liver enzymes. Livers from cetaceans suspected to have been exposed to oil should be analyzed for cytochrome P450, as well as hydrocarbon residues and other potential inducers, such as natural metabolites and organochlorines.

Table 8.3:1 Concentrations (in ng/mg microsomal protein) of cytochromes P450 and b_5, and total heme in fresh and stored liver from cetaceans and laboratory animals. Comparative data are provided from published reports.

Species	n	Storage Conditions		Total Heme	Cytochromes	
		Time	Temp.		b_5	P450
L. acutus	3	12-24 hrs.	0°C	0.85-1.72	0.29-0.48	0.34-1.77
T. truncatus	2	12 hrs.	0°C	0.59-0.73	0.24-0.48	0.19-0.23
P. phocoena	1	6 hrs.	0°C	0.99	0.43	0.57
Rabbit	3	fresh	--	2.9 -3.1	1.16-2.12	0.97-1.72
Rabbit	3	24-72 hrs.	0°C	2.0 -2.8	0.84-1.12	1.12-1.72
Rabbit	3	1-8 wks.	-20°C	2.23-3.47[a]	0.34-0.83	0.28-0.29
Rabbit	1	2 wks.	-70°C	3.29	1.17	2.26
Rat	2[b]	fresh	--	3.17	0.92	2.25
Rabbit[c]	-	fresh	--	2.55	1.12	1.55
Rat[d]	-	fresh	--	no data	no data	1.71
Baboon[e]	-	fresh	--	no data	no data	0.12
Man[f]	-	fresh	--	no data	no data	0.30

[a] n = 2
[b] pooled sample
[c] Omura and Sato 1965
[d] Booth and Gillette 1962
[e] Autrup *et al.* 1975
[f] Schoene *et al.* 1972

References

(1) Autrup, H., B.J. Thurlow, J. Wakhisi, and G.P. Warwick. 1975. Microsomal drug metabolizing enzymes in the Olive baboon. Comp. Biochem. Physiol. 50 (B): 385-390.

(2) Booth, J., and J.R. Gilette. 1962. The effect of anabolic steroids on drug metabolism by microsomal enzymes in rat liver. J. Pharmoid. and Exp. Therapeutics 137: p. 376.

(3) Lowry, O.H., N.J. Rosebrough, A.L. Farr, and R.J. Randall. 1951. Protein measurement with the folin phenol reagent. J. of Biological Chem. 193: 265-275.

(4) Omura, T., and R. Sato. 1964. The carbon monoxide-binding pigment of liver microsomes. J. of Biological Chem. 239: 2379-2385.

(5) Schilt, A.A. 1969. In: Analytical Applications of 1.10 Phenanthroline and Related Compounds. Pergamon Press. pp. 55-59.

(6) Schoene, B., R.A. Fleischann and H. Remmer. 1972. Determination of drug metabolizing enzymes in needle biopsies of human liver. Eur. J. Clin. Pharmacol. 4: 65-73.

8.4 Oil Ingestion in Rats

Introduction

We tested the rationale of the approach used for the previously described studies on metabolism and accumulation of crude oil in marine mammals in a brief laboratory study on rats.

Materials and Methods

Nine Wistar rats were divided into three groups, and treated as follows: (1) Control rats were maintained on a normal diet.

(2) Three rats were killed 66 hours after gastric intubation of 1 ml of Inter-Provincial crude oil, containing inherent naphthalene at a concentration of 1 to 5 mg/ml.

(3) Three rats were killed 40 hours after gastric intubation of 1 ml of Inter-Provincial crude oil which had been saturated with naphthalene (approximately 1 g/ml).

The rats were weighed and then killed by cervical dislocation. For each group, the livers from two rats were excised and weighed. Liver microsome isolation, and subsequent heme, cytochrome P450 and cytochrome b_5 analyses were performed as previously described.

Liver samples were taken from the remaining rat and processed for light microscopy. At the same time, liver, abdominal fat, and brain tissues were collected and analyzed for naphthalene as previously described. As a test of the naphthalene assay procedure, known amounts of naphthalene were added to homogenates of each tissue and the recovery efficiency was calculated.

Results and Discussion

Oil-dosed rats showed no overt behavioral reaction to the ingested oil; however those receiving the naphthalene-saturated oil were recumbent and lethargic. No pathological changes were noted on light microscopic examination of the liver in any of the rats, but the liver cells reacted to the oil biochemically by increasing concentrations of heme-containing cytochromes P450 and b_5 (Table 8.4:1).

Table 8.4:1 Microsomal analysis of liver from rats ingesting Inter-Provincial crude oil and naphthalene. Analyses were performed on blood samples from two rats in each group.

Microsomal Analysis*	Treatment		
	Control	Crude Oil	Crude Oil & Naphthalene
Total heme	20.8	30.3	45.8
Cytochrome b_5	6.07	8.67	12.0
Cytochrome P450	17.1	22.6	37.4

* Expressed in nmol/g liver

Naphthalene concentrations in liver, fat and brain of the oil-dosed rats did not differ from the controls (Table 8.4:2). When naphthalene-saturated oil was administered, higher naphthalene residues were detected in fat only.

This brief study has several important implications for bioaccumulation research on cetaceans. It demonstrates that ingested crude oil is a potent inducer of cytochrome P450, which likely plays a key role in the detoxification process. As such, analysis of cytochrome P450 in the livers of

dolphins stranded in the vicinity of an oil spill might indicate whether the animals had ingested oil. Secondly, since naphthalenes seem to be stored principally in fat, it may be assumed that blubber would be the target site of naphthalene storage in cetaceans. Fortunately, blubber is readily available for analyses, by simple biopsy of live animals.

Table 8.4:2 Naphthalene residues in tissues of rats ingesting Inter-Provincial crude oil and naphthalene.

Treatment	Tissue Naphthalene*		
	Liver	Fat	Brain
Control	< .07	< 1.0	< 1.0
Crude Oil	< .08	< 1.0	< 1.0
Crude Oil & Naphthalene	< .08	< 7.5	< 0.9

* in ppm

8.5 A STUDY OF THE EFFECTS OF OIL INGESTION ON A BOTTLENOSE DOLPHIN, TURSIOPS TRUNCATUS

From a Report Prepared by
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Introduction

In 1968, a Florida marine mammal facility experienced an unprecedented and unexplained increase in mortality of its bottlenose dolphins. The only consistent clinical feature of the affected animals was elevated serum activity of glutamic pyruvic transaminase (GPT), suggesting liver damage. Intensive investigation by the staff members and consultants failed to identify the cause of the problem. However, the coincidental occurrence of an accidental oil spill from hydraulic equipment into the holding tanks led the investigators to suspect that exposure to the oil might have caused the mortalities.

To test this hypothesis, small doses of refined oil were administered orally to a bottlenose dolphin. As a control, a second dolphin received mineral oil. The toxicity of the oil was also determined in a separate feeding trial using rats.

Materials and Methods

Two adult bottlenose dolphins were captured off northeastern Florida (Table 8.5:1) and acclimated for 1.5-4.5 months in a 6.6 m diameter circular tank containing pre-filtered, copper-treated sea water at ambient

Table 8.5:1 Basic data on experimental dolphins used in oil ingestion tests, both adults, at capture in northeastern Florida in 1968.

Animal Number	Standard Length (cm)	Sex	Date of Capture	Locality of Capture
Fed machine oil mixture:				
245	230	F	August 1	ca. 2 km N Crescent Beach
Fed machine oil as control:				
227	248	F	April 28	S of St. Augustine Beach

ocean temperature. Three or four times daily, the animals were fed a mixed diet consisting of two or more different species of fish, supplemented with multivitamin capsules and ascorbic acid. The food fish variably included blue runner (Caranx crysos), butterfish (Peprilus triacanthus), thread herring (Opisthonema oglinum) and herring (Clupea harengus).

Beginning September 13, 1968, dolphin 245 was fed a mixture of equal parts Sinclair Duro Oil #250 and Standard Oil RMP Delco supercharge 3 SAE10, at a rate of 5 ml per day, 5 days per week. The oil was given in two doses each day, by placing oil-filled capsules in the animal's food fish. By the end of the experiment on December 20, 1968, dolphin 245 had ingested oil at an average daily intake of 3.4 ml per day, for a total of 335 ml of oil (Table 8.5:2). Dolphin 227 received mineral oil on a similar schedule, from October 15 to December 20, 1968. This animal ingested an average of 3.3 ml per day, for a total of 225 ml of mineral oil (Table 8.5:3).

Blood samples were collected at various intervals throughout the ingestion study, and analyzed for activity of GPT. Hematology and blood urea nitrogen (BUN) were occasionally determined.

Three groups of six young adult Charles River rats received 0.5 ml of oil (approximately 65-95 ml/kg body wt) orally daily for fourteen days. One group received Sinclair Duro Oil #250, the second group received Standard Oil RPM Delco supercharge 3 SAE10, and the third group received cottonseed oil[1]. The rats were weighed daily. At the end of the two-week period all animals were sacrificed, a complete necropsy was performed, and histologic sections were taken from liver, kidney, spleen and pancreas.

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[1] Wesson oil

Table 8.5:2 Schedule for feeding machine oil by capsule to an Atlantic bottlenose dolphin, with cumulative amounts for the total experiment. (Note: A missing or partial date represents a weekend day or holiday when the technician was absent.)

DATE (1968)	VOLUME (ml)	CUMULATIVE VOLUME (ml)
September 13	5	5
16	5	10
17	5	15
18	5	20
19	5	25
20	5	30
23	5	35
24	5	40
25	5	45
26	5	50
27	5	55
30	5	60
October 1	5	65
2	5	70
3	5	75
4	5	80
7	5	85
8	5	90
9	5	95
10	5	100
11	5	105
14	5	110
15	5	115
16	5	120
17	5	125
18	5	130
21	5	135
22	5	140
23	5	145
24	5	150
25	5	155
28	5	160
29	5	165
30	5	170
31	5	175
November 1	5	180
4	5	185
5	5	190
6	5	195
7	5	200
8	5	205
11	2.5	207.5
12	5	212.5

Table 8.5:2 cont'd.

DATE (1968)		VOLUME (ml)	CUMULATIVE VOLUME (ml)
November	13	5	217.5
	15	5	222.5
	16	5	227.5
	18	5	232.5
	19	5	237.5
	20	5	242.5
	21	5	247.5
	22	5	252.5
	25	5	257.5
	26	5	262.5
	27	5	267.5
	29	5	272.5
December	3	5	277.5
	4	2.5	280
	6	5	285
	9	5	290
	10	5	295
	11	5	300
	12	5	305
	13	5	310
	16	5	315
	17	5	320
	18	5	325
	19	5	330
	20	5	335

Table 8.5:3 Schedule for feeding mineral oil by capsule to an Atlantic bottlenose dolphin, (#227) with cumulative amounts for the total experiment. (Note: A missing or partial date represents a weekend day or holiday when the technician was absent.)

DATE (1968)		VOLUME (ml)	CUMULATIVE VOLUME (ml)
October	15	5	5
	16	5	10
	17	5	15
	18	5	20
	21	5	25
	22	5	30
	23	5	35
	24	5	40
	25	5	45
	28	5	50
	29	5	55
	30	5	60
	31	5	65
November	1	5	70
	4	5	75
	5	5	80
	6	5	85
	7	5	90
	8	5	95
	11	2.5	97.5
	12	5	102.5
	13	5	107.5
	15	5	112.5
	16	5	117.5
	18	5	122.5
	19	5	127.5
	20	5	132.5
	21	5	137.5
	22	5	142.5
	25	5	147.5
	26	5	152.5
	27	5	157.5
	29	5	162.5
December	3	5	167.5
	4	2.5	170
	6	5	175
	9	5	180
	10	5	185
	11	5	190
	12	5	195
	13	5	200
	16	5	205

Table 8.5:3 cont'd.

DATE (1968)	VOLUME (ml)	CUMULATIVE VOLUME (ml)
December 17	5	210
18	5	215
19	5	220
20	5	225

Results and Discussion

Chronic ingestion of small amounts of hydraulilc oil or mineral oil did not have any clinically detectable effects on the two dolphins, with the possible exception of a three day period of inappetence beginning eight days after dolphin 245 began ingesting hydraulic oil. The results of all SGPT analyses were within normal limits established for these and other bottlenose dolphins (Table 8.5:4) (Ridgway 1972; Geraci and St. Aubin 1979).

One month after the ingestion study, dolphin 245 was euthanized for a separate study; dolphin 227 died following a stillbirth which occurred eight months after the experiment. Post-mortem examinations were performed on both dolphins. The dolphin ingesting hydraulic oil had extensive hepatic and pancreatic fibrosis, with hyperplasia of the ducts and infiltrations of mononuclear cells and eosinophils (Appendix 8.5:1). Trematode parasites were observed in the pancreatic ducts; these were considered to be responsible for the lesions in the pancreas and liver. No significant pathological findings were observed in dolphin 227.

Rats ingesting the low pressure Sinclair Oil grew at the same rate as the control rats (Figure 8.5:1). Those receiving Standard Oil had a mean growth rate that was slightly less than the controls, but was not felt to be significant by the investigating pathologist (Dr. J.C. Woodard, University of Florida). Histologic examination of the tissues did not reveal any morphologic alterations to suggest that the doses of oil administered were toxic.

The dolphin and rat studies demonstrated that small quantities of ingested hydraulic oil do not cause overt signs of toxicity. No liver damage was detected biochemically during the ingestion period, and histologic study failed to demonstrate any degenerative changes which

Table 8.5:4 SGPT values for Atlantic bottlenose dolphins used in oil ingestion experiments. The machine oil mixture was first fed on September 13, 1968. The mineral oil control was first fed on October 15, 1968. Horizontal lines indicate values for pre- and post-feeding of oil.

DATE	ANIMAL NUMBER	
	245 (fed machine oil)	227 (fed mineral oil)
31 July 1968		33
01 August 1968	25	
12 August 1968	45	
27 August 1968	43	
11 September 1968		38
13 September 1968	35	
26 September 1968	23	
11 October 1968		38
16 October 1968	40	
24 October 1968	28	28
05 November 1968	40	45
14 November 1968	45	40
26 November 1968	37	37
18 December 1968	23	40
30 January 1969	14	

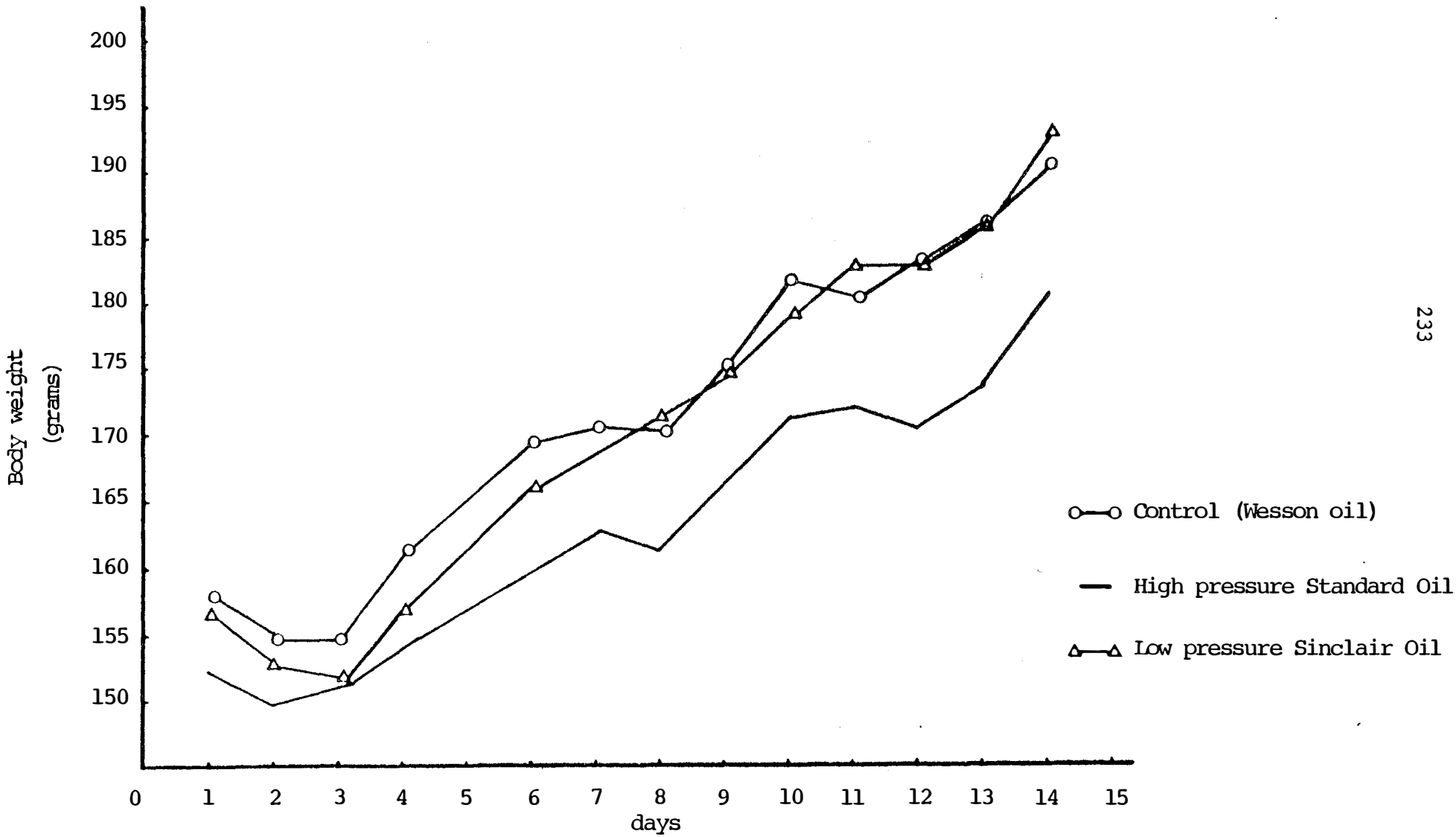


Figure 8.5:1 Mean body weight of three groups of six rats ingesting 0.5 ml/day of Standard Oil, Sinclair Oil or Wesson oil (control). Standard deviations for each data point are not available.

are usually associated with a toxic insult. No attempt was made to analyze tissues for petroleum residues to determine whether the dolphin accumulated and stored ingested oil. The investigators could not conclude that accidental ingestion of hydraulic oil was responsible for the previous unexplained dolphin mortalities.

References

(1) Geraci, J.R., and D.J. St. Aubin. 1979. Tissue sources and diagnostic value of circulating enzymes in cetaceans. J. Fish. Res. Bd. Can. 36 (2): 158-163.

(2) Ridgway, S.H. 1972. Homeostasis in the aquatic environment. In: Mammals of the Sea; Biology and Medicine. (Sam H. Ridgway, Ed.). Springfield, IL. Charles C. Thomas; 590-747.

Appendix 8.5 - Animal #245

HISTORY

Fed hydraulic oil mixture over a 3 month period (September 13 - December 20, 1968). Euthanized January 30, 1969.

GROSS FINDINGS

Externally the animal looked to be in excellent condition. In good fat condition and no notable cuts or scrapes.

Muscle: In good condition and no edema in the connective tissue, and no jaundice.

Respiratory System: Grossly normal. Some active lungworms and some old encysted lungworms.

Circulatory System: Grossly normal in appearance and color. Weight of heart 747 grams exclusive of auricles and some ventricular tissue. Main vessels grossly normal.

Digestive System: Teeth black, only the tips worn. No lesions in mouth or on tongue. Esophagus grossly normal. Forestomach contained partially-digested fish; grossly normal in appearance and no parasites. Fundic stomach grossly normal, empty and no parasites. Pyloric stomach grossly normal, empty and no parasites. Ampulla of duodenum grossly normal, empty and no parasites. Intestines grossly normal, containing liquid bile-stained feces.

Liver: Grossly normal. Rather whitish on surface, but good normal reddish brown color inside.

Spleen: Grossly normal. Typical reddish-whitish on outside and reddish on inside. Weight 63.0 grams. Several extra splenic nodules.

Pancreas: Grossly normal in overall appearance. Flukes found in main hepaticopancreatic duct.

Kidneys: Grossly normal.

Adrenals: Grossly normal. Right 11.0 grams, left 10.2 grams.

Urogenital System: Grossly normal. Left ovary with several corpora, right with none. Bladder normal.

HISTOPATHOLOGY

Kidney: No significant lesions.

Spleen: The spleen shows active lymphopoiesis, some of the central vessels of the Malphigian corpuscle are quite sclerotic. Large

quantities of hemosiderin are notably absent from the fibrous trabeculae and from the sub-capsular connective tissue. The capsule contains dilated vascular spaces which are presumed to be normal.

Heart: No significant lesions.

Liver: Fibrosis is rather extensive around the large biliary channels. There is also some proliferation of bileductules. The liver parenchyma itself shows no significant alteration except for some degree of biliary fibrosis in the portal triads; this space is also infiltrated with lymphoid cells.

Pancreas: Interstitial fibrosis is evident within the pancreas. Parenchymal degeneration is minimal but there is marked fibrosis around the large pancreatic ducts. There is proliferation of the pancreatic ductules and marked infiltration of the large ducts with massive numbers of eosinophils. Trematoparasites are present in the lumina. About some of the smaller bile ducts there is a foreign body type reaction and the formation of lymphoid follicles.

Lung: Chronic inflammation and interstitial fibrosis are evident within lung parenchyma. There is a minimal amount of exudation of fluid in cells into the alveolar spaces, but some edema fluid is present. Sub-luminal calcification in the respiratory bronchioles is also present.

ANATOMICAL DIAGNOSIS

(1) Hepatic and pancreatic trematodiasis.

(2) Chronic pneumonitis, possibly parasitic.

9.00 BALEEN FOULING

Introduction

Cetacean skin is smooth and essentially hairless, and oil in the marine environment is not likely to adhere to the outer body surfaces of these animals. However, the hair-like fringes of baleen plates can become coated if exposed to oil even briefly. The consequences of baleen fouling are unknown, but any alteration in the structure or function of baleen may interfere with normal feeding.

Mysticetes employ three basic feeding strategies (Pivorunas 1979). The rorquals, which include the blue, fin, minke, Bryde's, sei and humpback whales, capture their prey by engulfing a mixture of food and water, then forcing the water through the baleen filter which retains the food organisms. The right and bowhead whales are grazers, swimming through surface concentrations of plankton with their mouths open and passing a continuous stream of water through the baleen plates. The gray whales feed on benthic organisms, which are scooped up from the bottom along with mud and pebbles.

Irrespective of the feeding strategy, the filtration properties of baleen depend on an intricate network of fibers which project from the medial aspect of each plate to form a net that entraps food organisms within the whale's mouth. The fibers are held in a keratinized matrix which forms the body of the plate, but become exposed as the matrix is worn away by the tongue. Since the structure and function of baleen plates are so closely linked, our assessment of the consequences of oil fouling deals with both properties.

9.1 Baleen Function

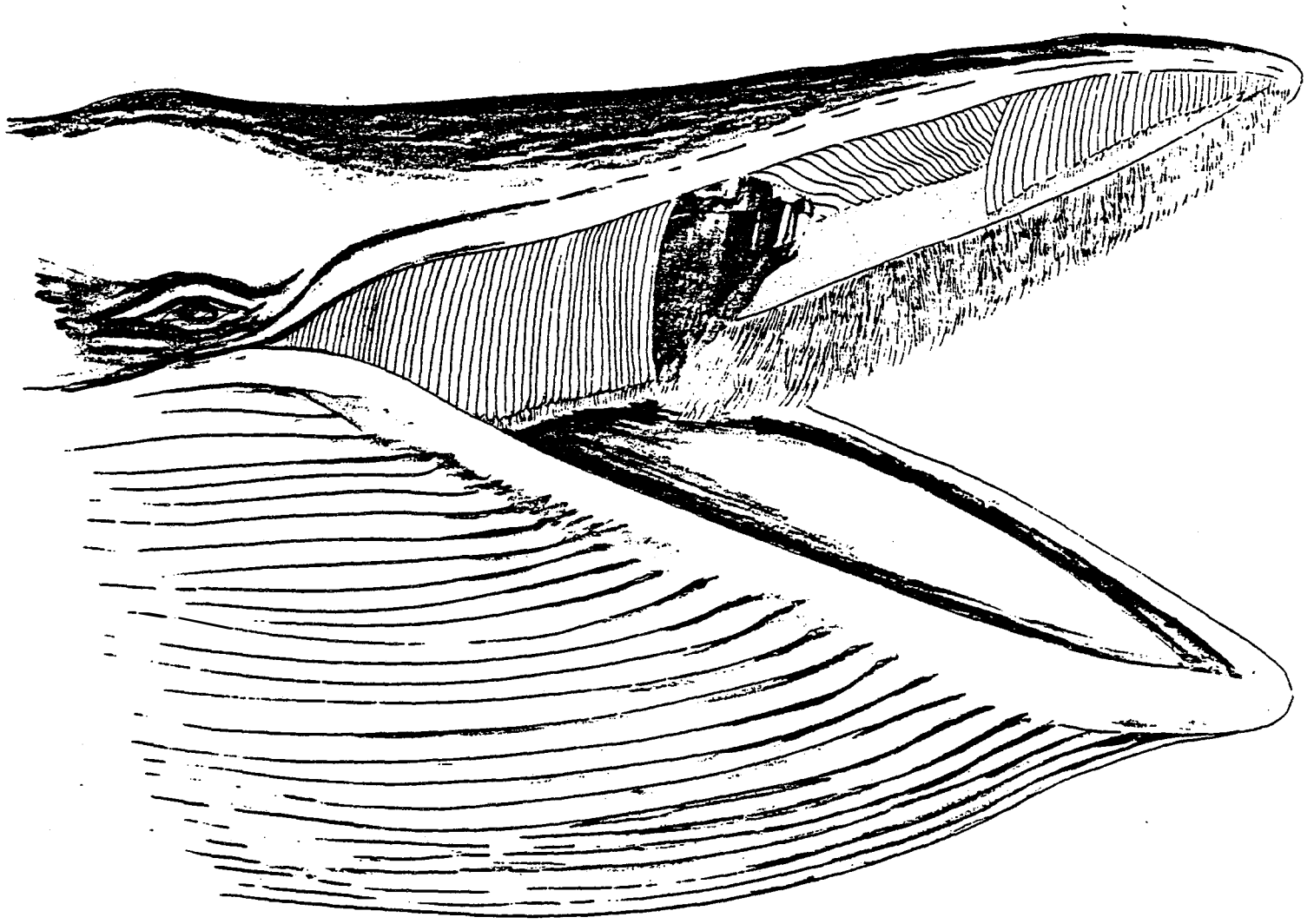
Materials and Methods

An *in vitro* study was conducted to assess the effects of exposure to various oils on the functional properties of baleen. Baleen samples from 6 species of whales were obtained from strandings along the coasts of California, New England and Nova Scotia, and from the Icelandic whale fishery (Hvalur H.F., Hvalfjordur, Iceland). The baleen sections were removed with the plates embedded in gum tissue, (Figure 9.1:1) and stored frozen.

Baleen sections were tested in an open channel water flume (Theakston) 1975) (Figure 9.1:2). The flume consists of a glass-sided channel measuring 4.7 m long, 92 cm wide, and 45 cm deep. Water from an in-ground 27,000 L reservoir is pumped continuously into an above-ground 2,300 L reservoir at one end of the flume. The resulting head produces a constant flow of water through the channel. At the other end, the water flows into a sump from which it returns by gravity feed into the main reservoir below. The apparatus can accommodate the use of both fresh and salt water at ambient temperatures ranging from 15°C to 21°C.

The inlet to the flume is 9 times the cross-sectional area of the observation area and is tapered to achieve a streamline flow. A gate at the lower end of the flume controls the water up to a depth of 40 cm; a valve on the pump intake controls the velocity of water upstream of the baleen within a working range of 5-15 cm/sec. The rate of flow between the baleen plates ranged between 20 and 30 cm/sec.

In operation, a section of baleen measuring up to 90 cm along the axis of the gum was mounted in a plexiglass frame so that the plates were suspended in the natural hanging position in the channel, or inverted with gum



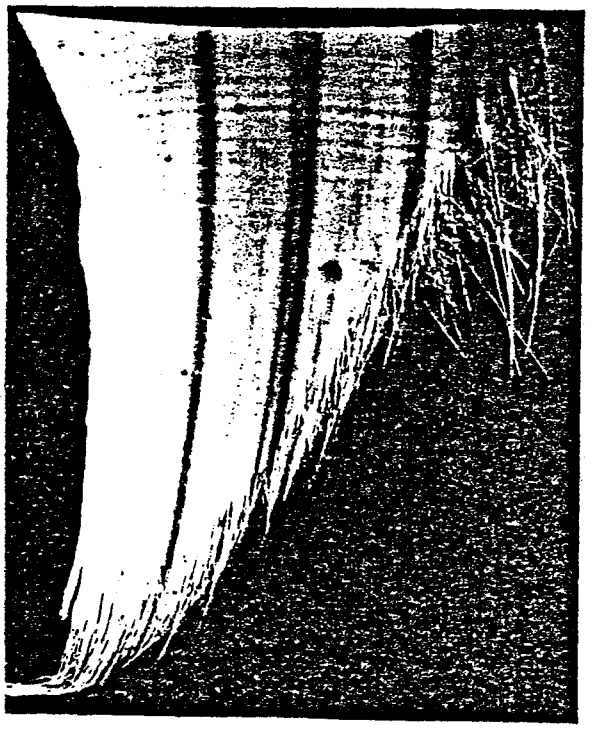
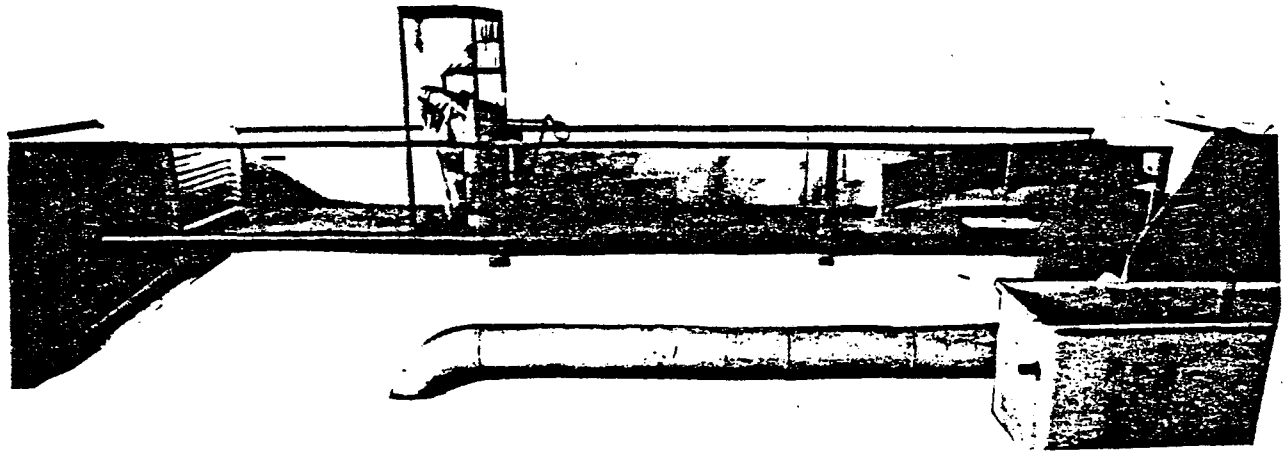
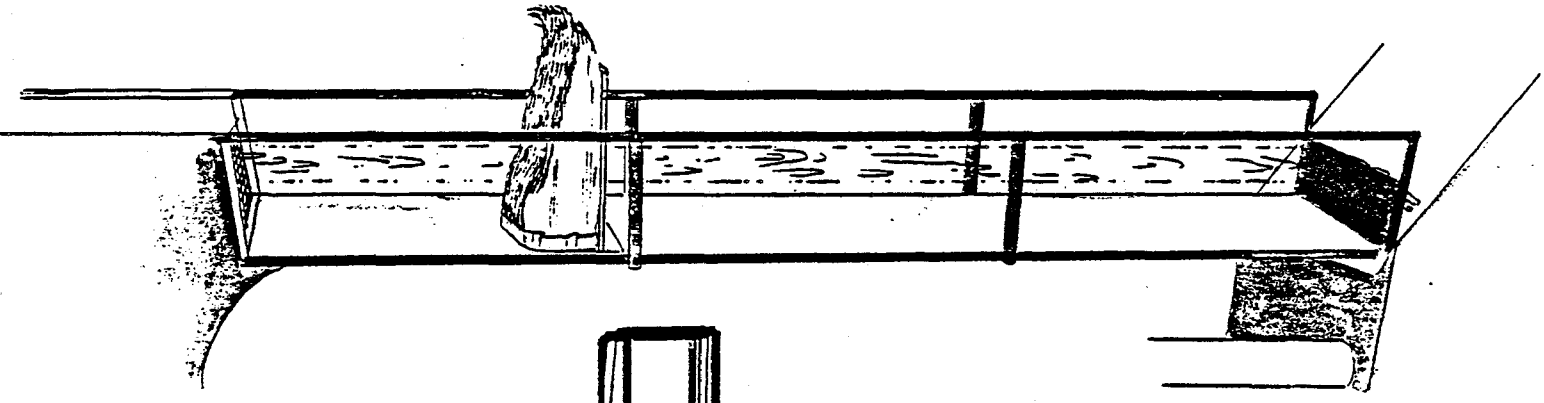
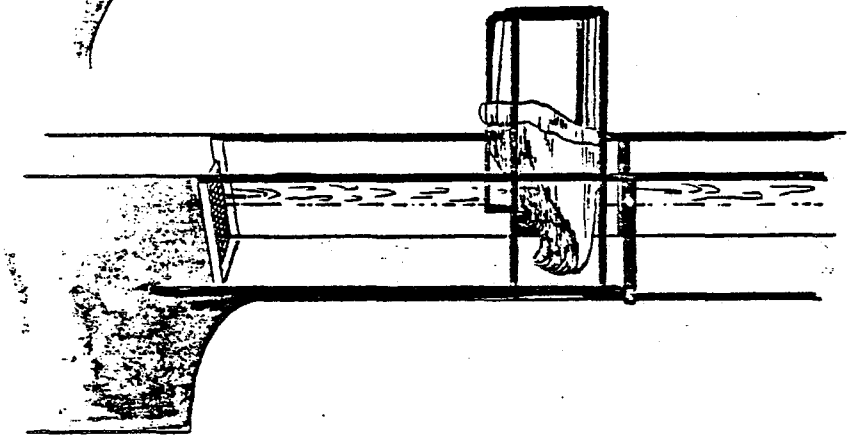


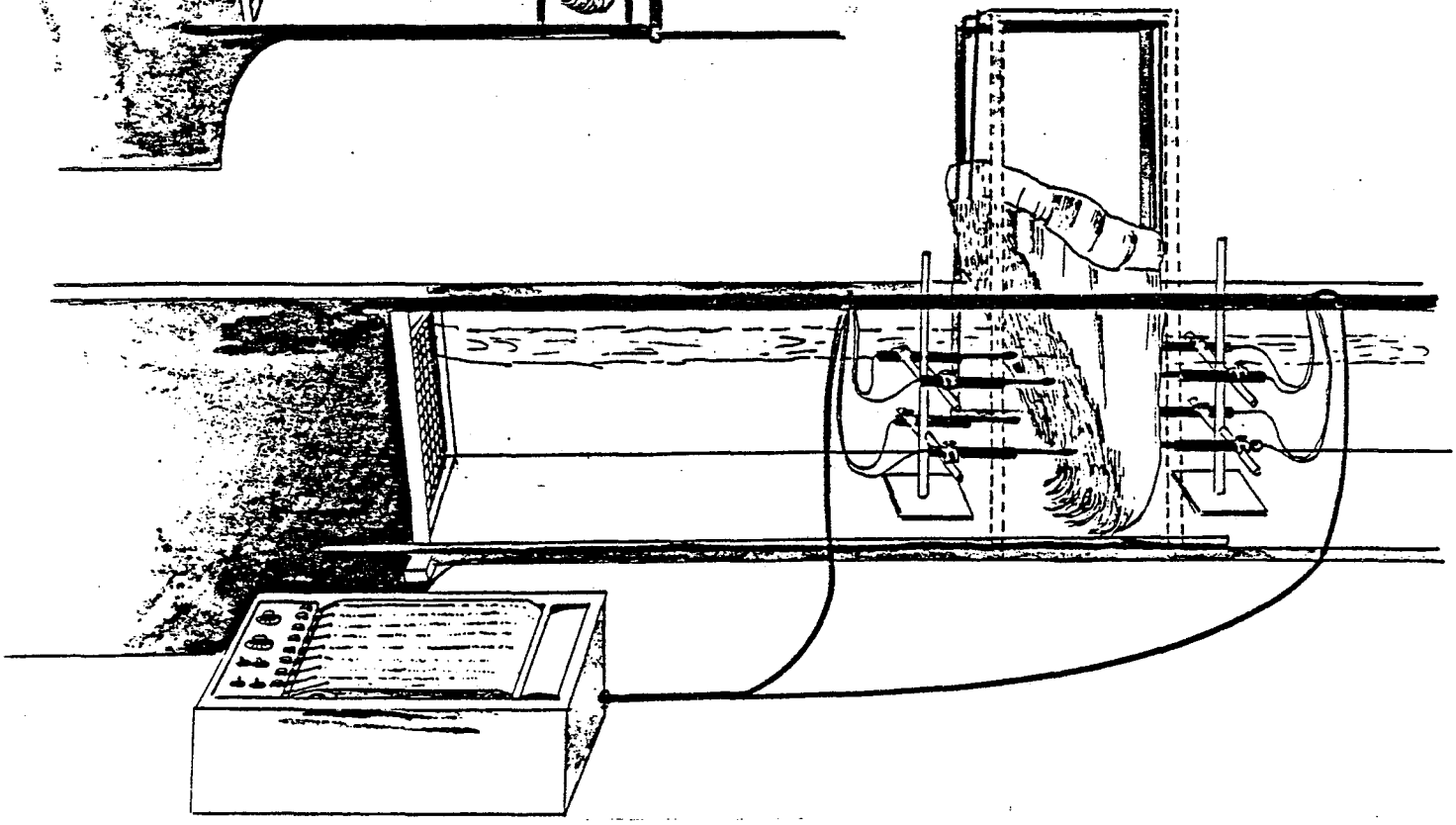
Figure 9.1:1 Samples of baleen are taken with the plates embedded in gum tissue. Individual plates are isolated for structural studies.

Figure 9.1:2 An open channel water flume is used to study the dynamics of filtration in unfouled and oil-fouled baleen preparations. A row of baleen plates is mounted in either of two modes: resting on the gum tissue, or suspended in the natural hanging position. Direction of the water flow is from left to right. Sensitive thermistor probes are placed on either side and between the plates to measure water flow, and the voltage output recorded on a multi-channel recorder.









tissue resting on the floor of the channel. In either mode, the baleen section was oriented so that water flowed from the medial (lingual) to the lateral (labial) surface, coming in contact first with the filter net in a way that simulated the water expulsion/food retention phase of feeding. The experimental apparatus was designed to duplicate the mechanism by which baleen plates normally function, and also enabled selective testing of proximal (nearest gum) or distal tips of the baleen plates for oil fouling effects.

Water movement was measured simultaneously in the incurrent and excurrent sides of the preparation as well as between baleen plates, using 4-8 hot bead thermistor flowmeters which could measure flows from 0.2 to 50 cm/sec. with a response time of about 200 ms and a spatial resolution of about 1 mm (La Barbera and Vogel 1976). The output of each flowmeter approximates a logarithmic function of the ambient water velocity. The signal from each flowmeter was independently recorded on an eight-channel cart recorder[1]. This allowed for instantaneous and continuous monitoring of the water movements in and around the baleen plates. The flowmeters were calibrated using an Ott current meter[2].

Once the flow patterns through each preparation were determined, control perturbations, such as dumping 4-20 L of water, inducing waves, or obstructing portions of the fringed surface of the plates with screening or fiberglass air filters, were performed to ensure that the flow sensors were functioning correctly (Figure 9.1:3). The baleen preparation was then fouled with oils of varying consistencies, either by direct application or by being churned into the water column, thus simulating a more natural exposure.

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[1] Narco Life Science

[2] Ott, Kempten, West Germany

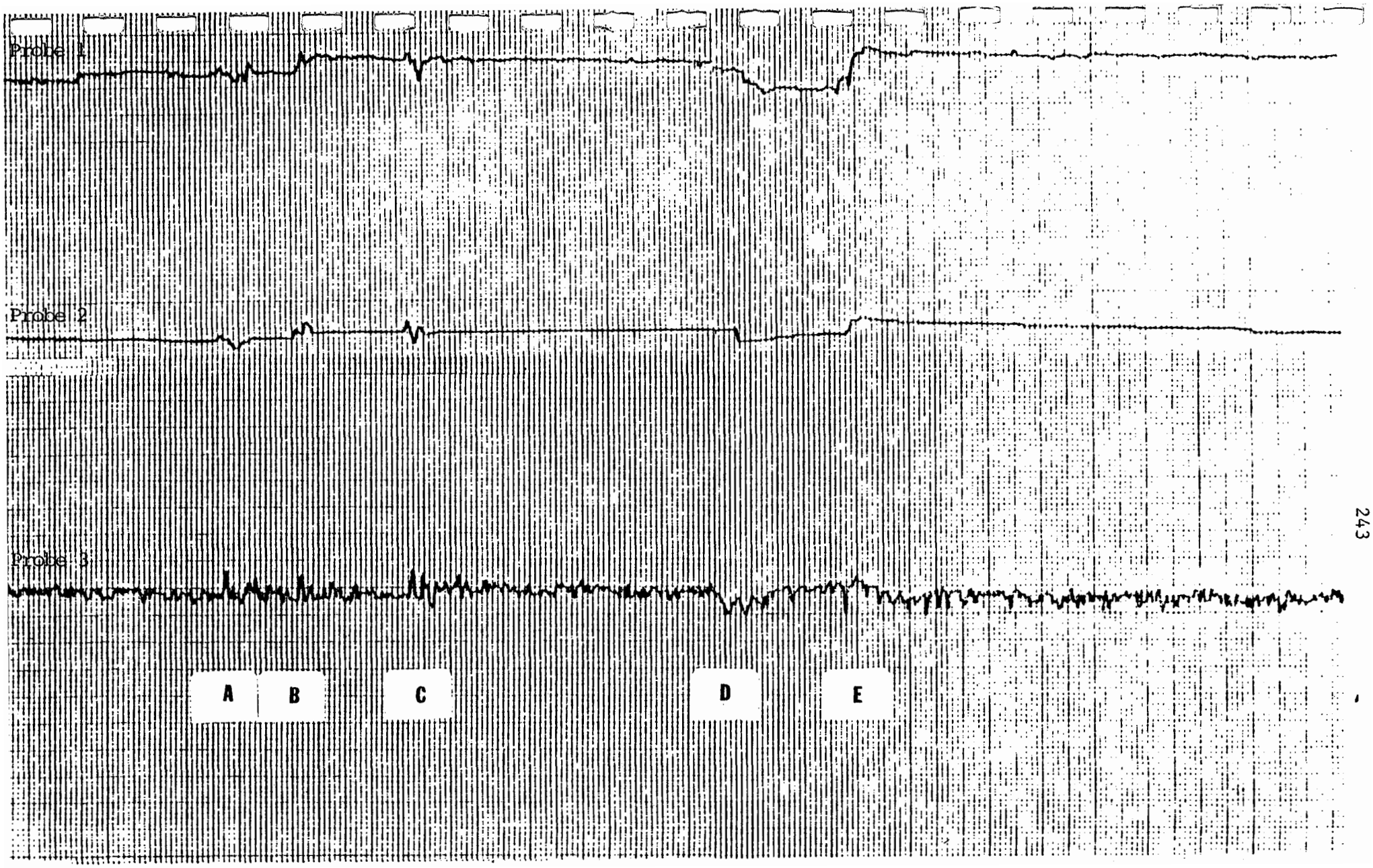

Figure 9.1:3 Control perturbations of water flow, such as inducing waves (A,C) or pouring water (B), performed prior to the introduction of oil. Obstruction of flow using an air filter on selected portions of the baleen (D-E) results in a variable depression of the flowmeter signal, reflecting localized disruptions. 1 cm = 1 sec.

The oils used were Inter-Provincial crude (a medium weight mixture of heavy and light crudes), West Texas crude (a relatively volatile, low viscosity oil), Bunker C, and roofing tar. Up to 3 foulings of 4 L each were performed on each baleen preparation.

Three baleen specimens (2 fin whale, 1 gray whale) which were fouled with Inter-Provincial crude or Bunker C during functional tests in the flume were placed in a closed system with flowing salt water (30-40 g L^{-1} NaCl) to monitor the cleansing rate of the fibers. The testing chamber consisted of a hexagonal fiberglass tank with a weighted rectangular centerpiece (Figure 9.1:4). The baleen specimens were suspended in their natural orientation in a current produced by a pump delivering approximately 500 L/min. A skimming device confined all of the oil which was removed from the baleen, to prevent re-fouling.

Three samples of 10-20 fibers were taken prior to placing each section of baleen into the tank, and at 1-2 hour intervals during the first 12 hours of the cleansing process. Thereafter, samples were collected at irregular intervals for up to 2 days. The oil-fouled fibers were air dried, weighed, repeatedly soaked in 10 ml aliquots of HPLC-grade hexane to remove the oil, air dried and reweighed. The difference in weight represented the amount of oil which had adhered to the fibers, and endogenous lipids extracted from the fibers. Using un-oiled specimens, we determined that 3-11% of the fiber weight was removed by the hexane. This correction was applied to all determinations on oiled fibers. Results were calculated as the ratio of oil weight to fiber weight, and expressed as a percentage.

The amount of oil in the hexane rinsate was quantified photometrically using a Perkin Elmer Model 55 spectrophotometer[1], equipped for UV

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[1] Perkin Elmer Corp., Maywood, IL

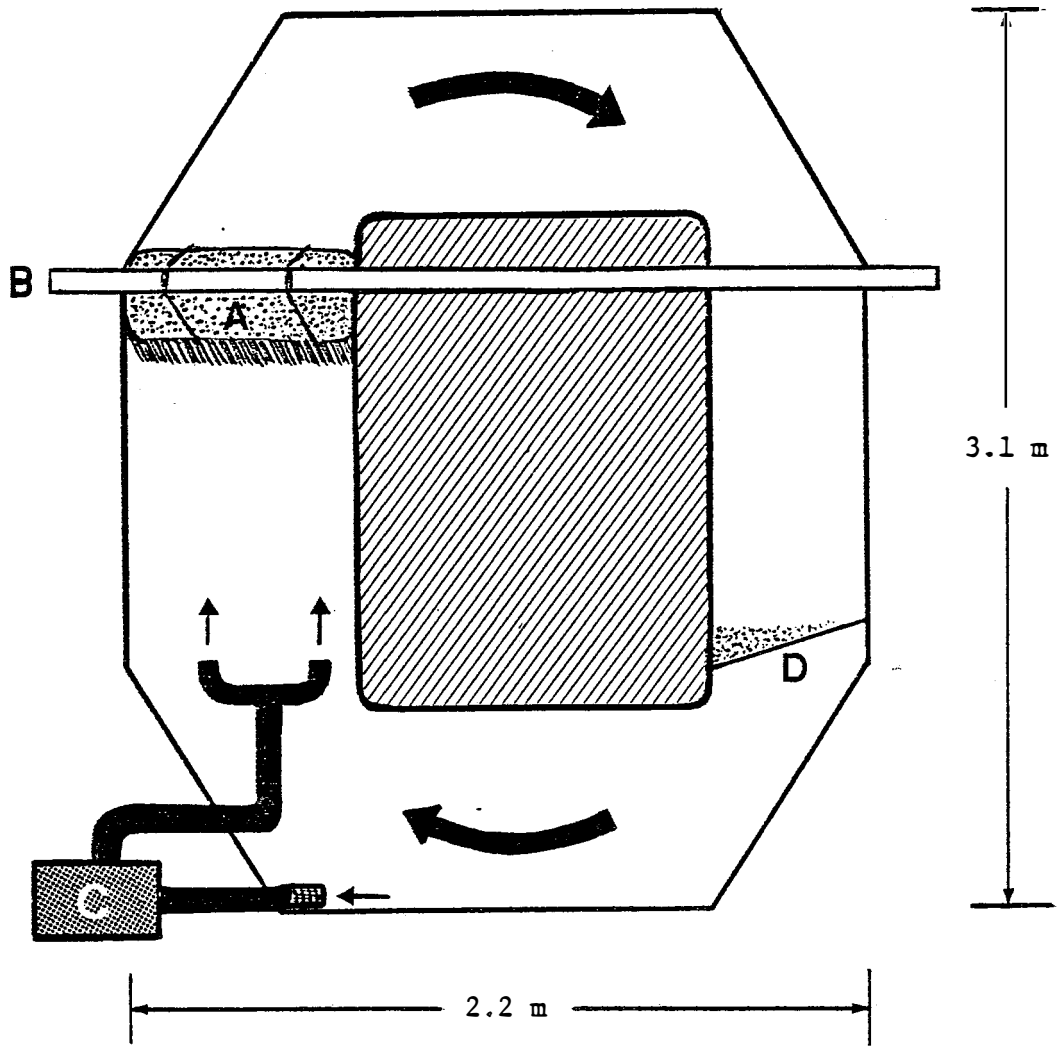


Figure 9.1:4 Apparatus for monitoring the cleansing of oil-fouled baleen. The baleen (A) was suspended in its natural hanging position from a supporting beam (B). A pump (C) maintained continuous circulation (arrows) of salt water (3-4%) through the plates, from the medial to the labial surfaces, and a skimmer (D) entrapped oil to prevent refouling of the baleen.

operation. Fresh and weathered samples of Inter-Provincial crude oil and Bunker C were analyzed using a Pye-Unican SP8000 scanning UV spectrophotometer, to determine the absorbance characteristics of each between 230 and 450 nm. Wavelengths chosen to quantify the oil in each hexane rinsate were 250-256 nm for Bunker C and 325 nm for Inter-Provincial crude. Standard curves relating concentration to absorbance were prepared for each type, and extinction coefficients (ε) were calculated using the formula:

$$A = \varepsilon C l$$

where A = absorbance

C = concentration of oil

l = light path

= 1 cm

The values for ε obtained for Inter-Provincial crude and Bunker C were 0.000146 mg^{-1} L cm and 0.031 $\pm$ 0.001 L/mg^{-1} cm respectively, at the chosen wavelengths.

Results and Interpretation

Eighteen trials were run on fin whale baleen, and two on gray whale baleen. Oil effects on the portion of the plates nearest the gum were investigated in eleven of the fin whale samples; (Figure 9.1:5a), seven trials were run on the distal fringes only (Figure 9.1:5b). The comparatively short gray whale baleen plates were studied as a single unit (Figure 9.1:5c).

Inter-Provincial and West Texas crude oils caused an immediate but transient change in the pattern and rate of water flow, which was more apparent in the fin whale baleen than in the gray whale specimen (Figure 9.1:6-9.1:8). The passage of oil through the baleen produced a local reduction in the flow rate, likely due to the higher viscosity of the oil. Compensatory increases in flow were detected by probes placed in portions of the baleen not exposed to the main concentration of oil (Figure 9.1:6). The duration of the altered flow patterns was 5-40 sec.

The most pronounced depression in water flow occurred in the portion of the fin whale baleen closest to the gum (Figure 9.1:6), as a probable function of the density of fibers in this area. The gray whale specimen presented the least resistance to water flow, since the plates are more widely spaced, and the medium weight crude oil produced little disruption in the rate or pattern of flow (Figure 9.1:7). Distal portions of the fin whale plates were similarly less affected (Figure 9.1:6).

Bunker C and roofing tar caused a much more dramatic reduction in water flow. The increased resistance to flow was apparent as a change in the water level upstream from the fouled baleen. The effect lasted 10-15 minutes, after which normal flow patterns were restored, though the hairs remained oil-fouled.

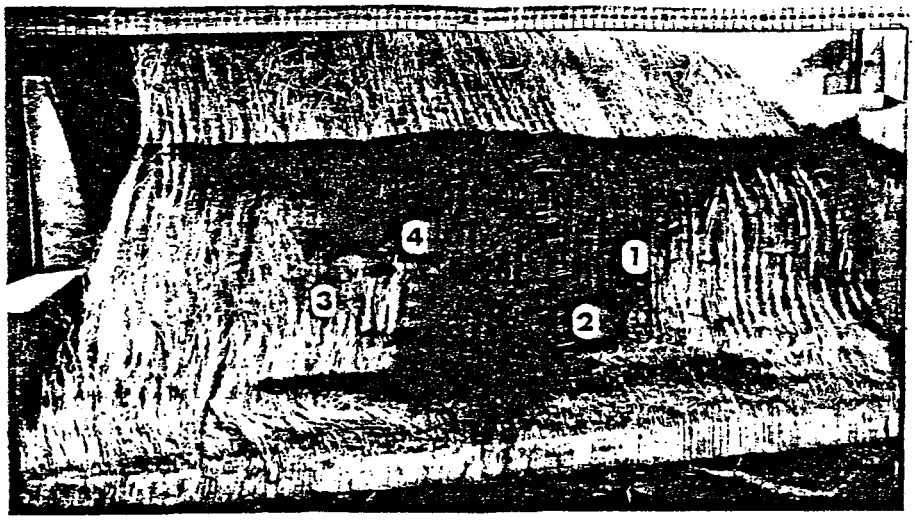


Figure 9.1:5a Oiled fin whale baleen, tested in the inverted, or gum-down, position. Numbers correspond to the position of flowmeters (see Figure 7.1:5).

Figure 9.1:5b Oiled fin whale baleen. The fringes were selectively fouled in this trial.

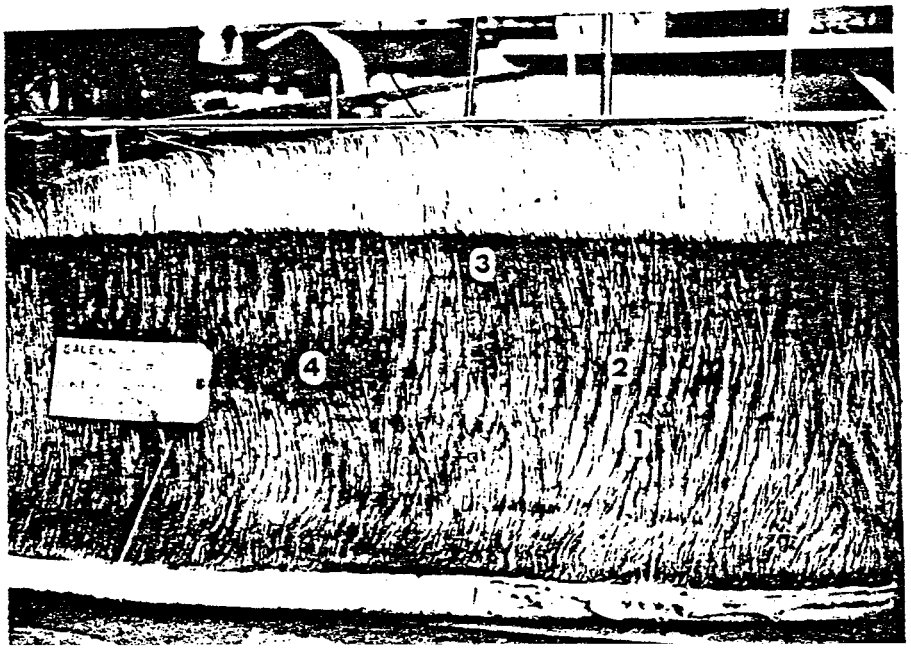


Figure 9.1:5c Oiled gray whale baleen. The whole specimen was fouled at once, due to the relatively short plates. Numbers correspond to the position of flowmeters (see Figure 7.1:7).

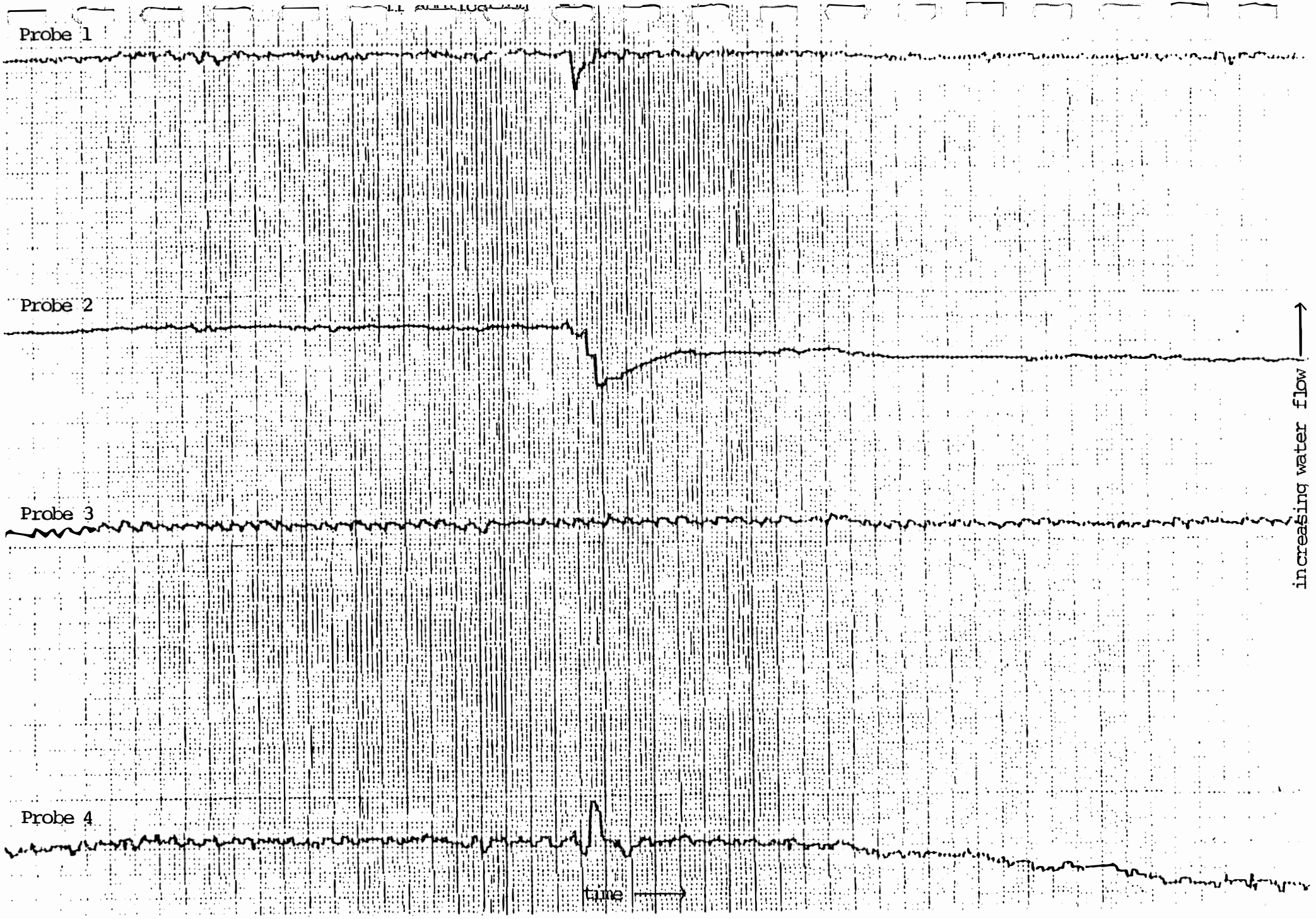


Figure 9.1:6 Effects of 4 L of Inter-provincial crude oil on the proximal portions of fin whale baleen. The positions of the four flowmeters are shown in Figure 7.1:3a. 1 cm = 10 sec.

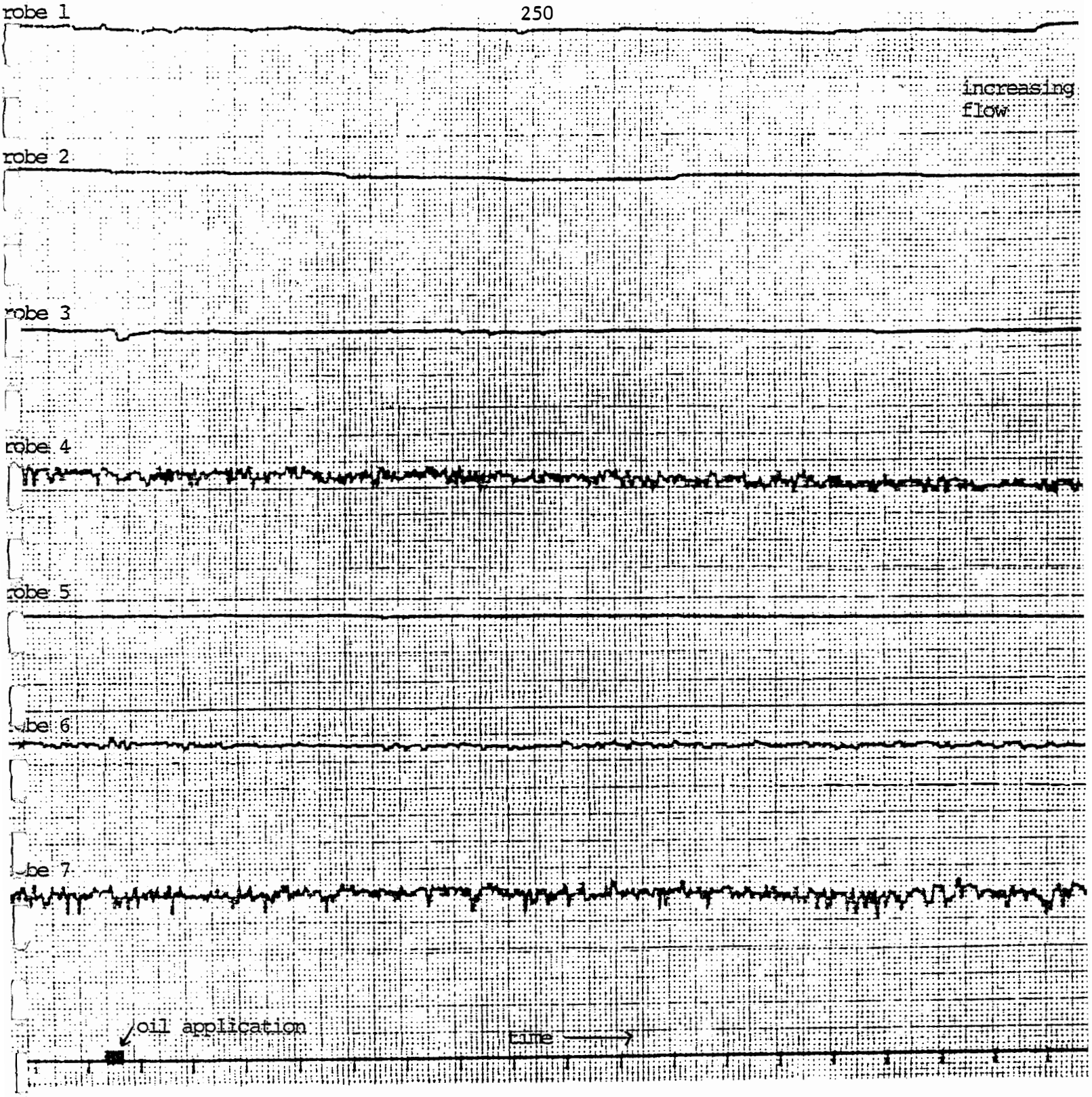
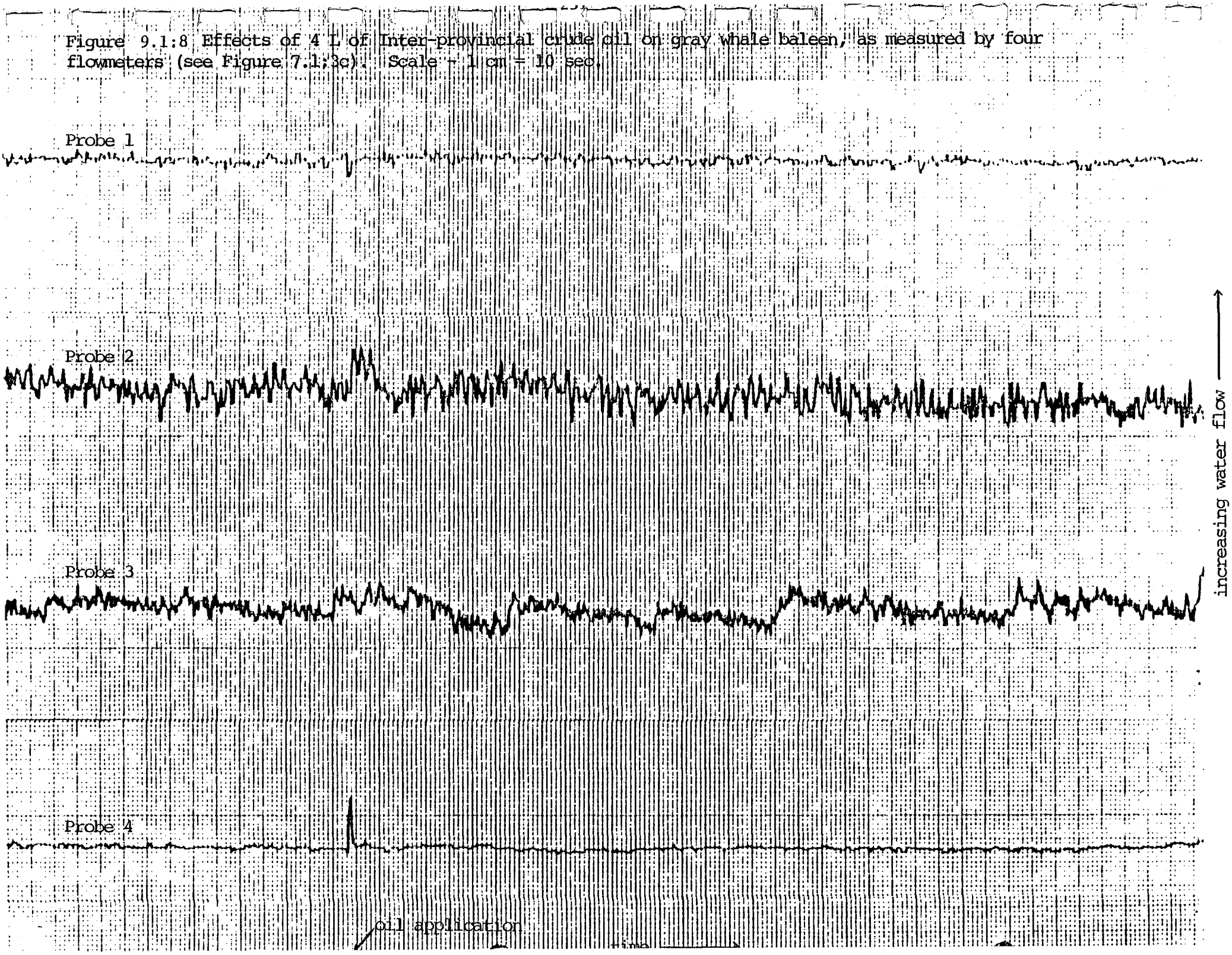


Figure 9.1:7 Effects of 4 L of Inter-provincial crude oil on the distal fringes of fin whale baleen. The event marker on the baseline indicates the application of oil. 1 cm = 1 sec.

Figure 9.1:8 Effects of 4 L of Inter-provincial crude oil on gray whale baleen, as measured by four flowmeters (see Figure 7.1:3c). Scale - 1 cm = 10 sec.



To test the effects of repeated exposure on the same section of baleen, seven trials where run in which oil was applied two or three times, at 10-15 minute intervals. The same alterations in flow were observed with each application, and the effects were not additive.

Two fin whale specimens fouled with Bunker C and one gray whale specimen fouled with Inter-Provincial crude were rinsed continuously in salt water for up to two days to monitor the recovery of the baleen. The combined data for the fin whale show a rapid and marked decrease in the amount of oil adhering to the fibers (Figure 9.1:9). Spectrophotometric analysis of the oil eluted from the fibers showed a similar pattern, with an even shorter time course. Gray whale baleen fouled with the more volatile Inter-Provincial crude oil had the fastest recovery time. The oil was undetectable by weighing after 1 hour of flushing, though trace amounts representing approximately 1.5% of the fiber weight could still be detected spectrophotometrically, after 30.5 hours. The disappearance of the oil from all specimens was readily observed.

The studies suggest that baleen function would be transiently affected by oil, particularly heavy, weathered fractions. At 15°C, the effect may be reversed within an hour for the lighter oils, and 15-20 hours for more viscous substances such as Bunker C. In colder water these effects may be somewhat more persistent.

References

(1) La Barbera, M. and S. Vogel. 1976. An inexpensive thermistor flow-meter for aquatic biology. Limnology and Oceanography 21: 750-756.

(2) Pivorunas, A. 1979. The feeding mechanisms of baleen whales. Amer. Scientist 67 (4): 432-440.

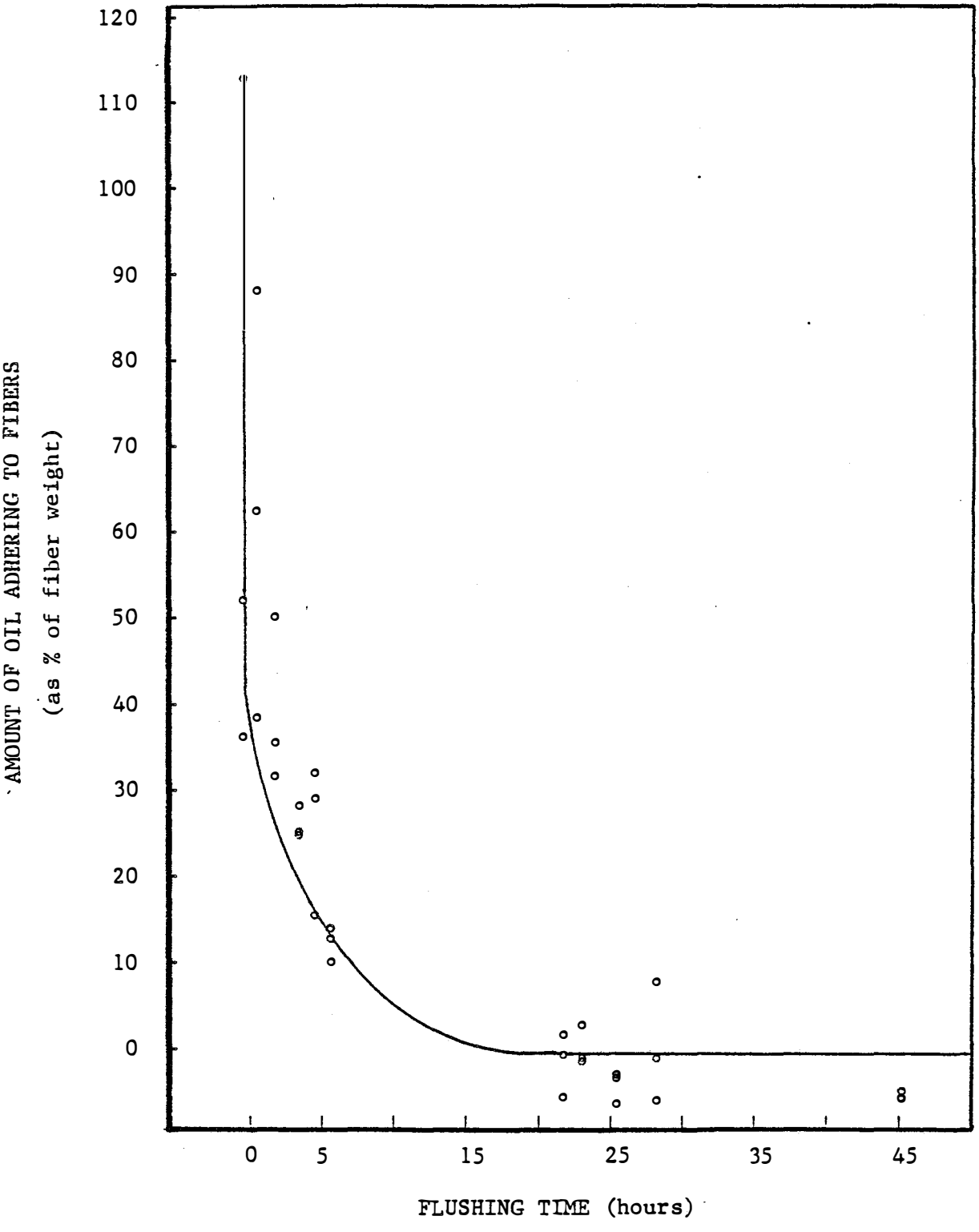


Figure 9.1:9 Loss of Bunker C oil from the fibers of fin whale baleen continuously flushed in salt water. The curve was visually fitted.

9.2 Effects of Petroleum on Structural Properties of Baleen

Introduction

Oil fouling of baleen may produce structural changes in one or more components of the plates, resulting in aberrant wear patterns, loss of integrity, or breakage, and thereby interfere with normal function. Volatile oil fractions, in particular, are destructive to tissue, and may ultimately have the greatest impact on the feeding mechanism of mysticetes.

We assessed the effect of petroleum hydrocarbons on isolated baleen plates by soaking them for various periods of time. The exposure periods exceeded those which might naturally occur, in order to exaggerate any changes which might otherwise be difficult to detect. Plates were also treated with a decalcifying agent, trichloroacetic acid (TCA), to assess the role of calcium in the structure of baleen. The integrity of the treated plates was determined by tensiometry, x-ray diffraction and elemental analysis.

Materials and Methods

Samples and Treatments

Baleen samples obtained from six mysticete species were subjected to various treatments and analyses as detailed in Table 9.2:1. The plates were removed at the gumline, and placed into saline (3.0%) baths for 24-96 hours to reverse any dehydration which might have occurred during frozen storage of the samples. Individual plates from one fin, gray, and right whale were then transferred to one of the treatment baths as follows:

Table 9.2:1 Treatments and analyses performed on isolated baleen plates

Species	No. Individuals	Total No. of Plates	Analyses	No. of Plates Treated				
				Control	Gasoline	Crude Oil	Tar	TCA
fin	2	17	Tensiometry	9	1	1	1	1
			X-ray Diff.	2	2	1	1	1
			Composition	3	2	1	1	1
gray	2	11	Tensiometry	2	-	-	-	-
			X-ray Diff.	4	1	1	2	1
			Composition	3	2	1	2	-
right	1	12	X-ray Diff.	3	3	3	2	1
			Composition	3	3	3	2	-
minke	1	1	X-ray Diff.	1	-	-	-	-
			Composition	2	-	-	-	-
humpback	1	3	X-ray Diff.	1	-	-	-	-
			Composition	2	-	-	-	-
sei	1	2	Tensiometry	1	-	-	-	-
			X-ray Diff.	1	-	-	-	-
			Composition	1	-	-	-	-

Treatment	Time
lead-free gasoline (Esso 2000)	1.5 hours or 14 days
West Texas crude oil	8 hours
roofing tar (Domtar)	21 days
decalcifying solution (10% TCA)	8 days

After treatment, the plates were wiped dry and processed for tensiometry, x-ray diffraction, or elemental analysis. Plates soaked in tar had to be cleaned using small amounts of gasoline.

Tensiometry - Methods

The procedure employed for structural tests of baleen plates was derived from a method described by the American Society for Testing of Materials (ASTM) (Anon. 1973). One of two templates, with dimensions conforming to specifications described by the ASTM (Figure 9.2:1) was placed on the baleen plate at prescribed distances from the gumline. The template was outlined with a wax marker, thereby describing the shape of the pieces to be cut out and tested (Figure 9.2:2). The tubules within the keratinized matrix were aligned perpendicularly to the long axis of the test piece, so that a force applied to each end tended to part the matrix without breaking the tubules.

The test pieces were soaked in saline (3.0%) prior to testing. The breaking strength and elasticity of each piece was determined using an Instron tensiometer[1] equipped with chart recorder. Calculation of tensile strength is based on minimum cross-sectional area, determined

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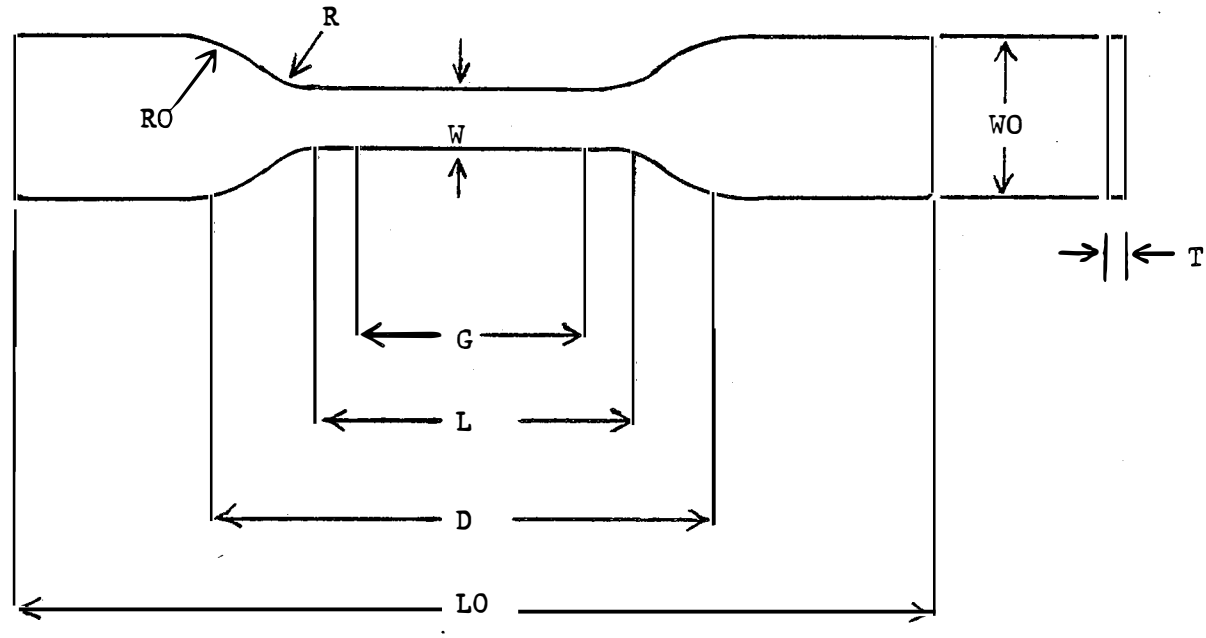
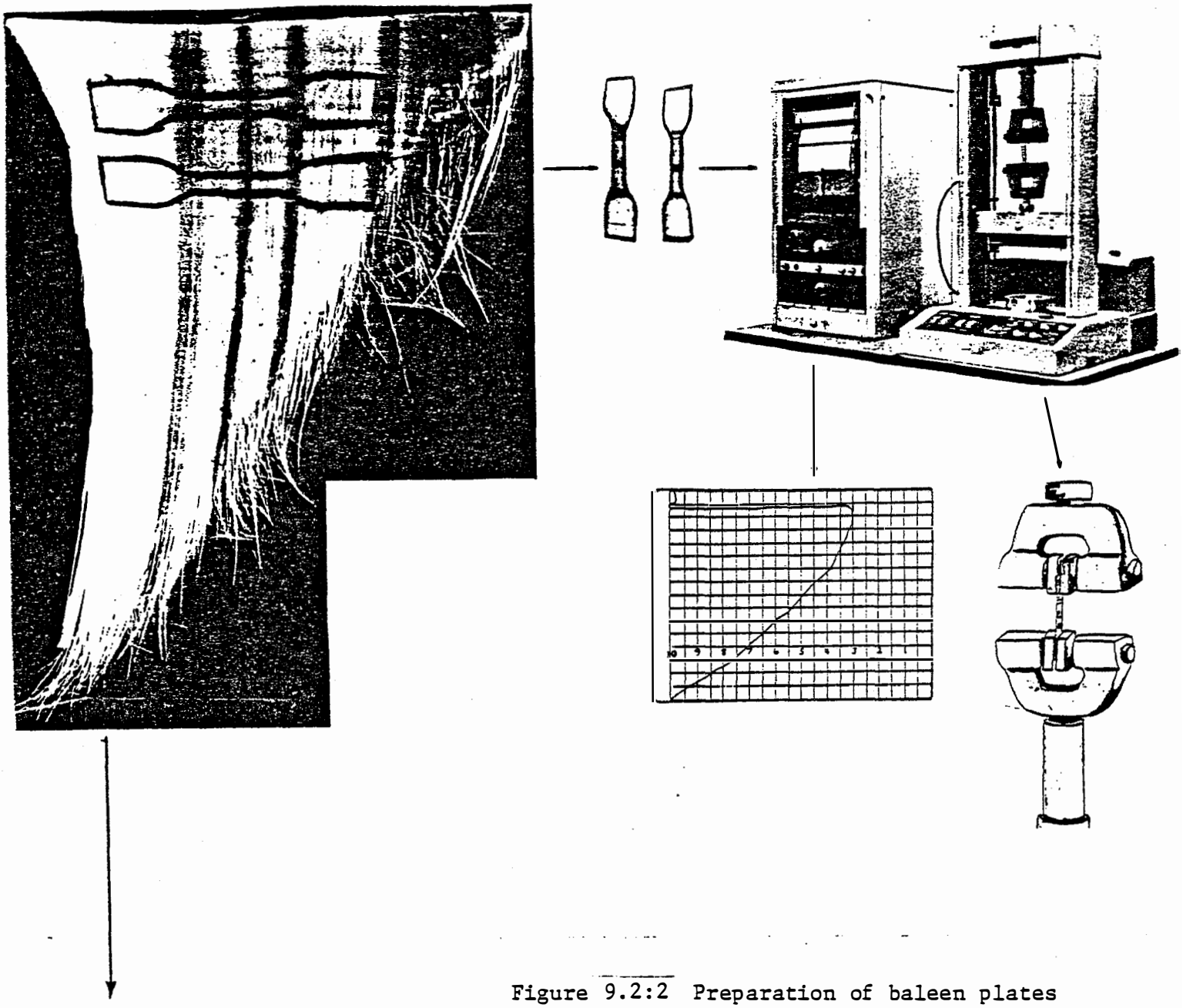
[1] Instron Corporation, Canton, MA

Figure 9.2:1 Template specifications for preparation of baleen samples for tensiometry.



Measurement	Dimensions (mm) Template IV	Template V
W - Width of narrow section	6.0 ± 0.5	3.18 ± 0.5
L - Length of narrow section	33.0 ± 0.5	9.53 ± 0.5
WD - Width over-all	19.0 ± 6.0	9.53 ± 6
LO - Length over-all, min	115 (no max)	63.5 (no max)
G - Gage length	25.0 ± 0.13	7.6 ± 0.25
D - Distance between grips	64.0 ± 5.0	25.4 ± 5
R - Radius of fillet	14.0 ± 1.0	12.7 ± 1
RO - Outer radius	25.0 ± 1.0	-
T - Thickness	less than 4	less than 4



Chemical Analysis
X-Ray Diffraction

Figure 9.2:2 Preparation of baleen plates for studies on structural integrity. Samples across the plates are cut according to ASTM specifications, and tested for tensile strength using an Instron tensiometer. A chart recorder displays breaking strength and elasticity (top right). Sub-samples of the plate are taken for chemical and x-ray analysis, as well as microscopy.

using calipers, and maximum load as follows:

$$\underline{\text{Stress}} = \text{tensile strength } (\sigma) = \frac{\text{maximum load}}{\text{minimum area}}$$

The test procedure also measures strain, defined as the change in length of the sample prior to breaking, in relation to its total length, as follows:

$$\underline{\text{Strain}} \quad (\varepsilon) = \frac{\text{change in length}}{\text{original length}} = \frac{\Delta L}{L}$$

$$= \frac{\text{grip speed x time to breakage}}{\text{distance between grips}}$$

$$\underline{\text{\% Strain}} = \text{proportional increase in length of the test piece}$$

$$\frac{\Delta L}{L} \times 100\%$$

Only baleen samples from fin, gray, and sei whales were tested (Table 9.2:1), since the plates available from the right, humpback, and minke whales were too narrow. The ratio of stress to strain, defined as Young's modulus, provides a measure of the strength of the sample.

X-ray Diffraction - Methods

Samples were cut from medial and lateral portions of the baleen, both at the gumline and distally at a level which is 3/4 of the total length of the plate. Fibers were also taken from this level. Pieces of baleen 1 mm x 1 mm, or individual fibers, were mounted on plasticene and exposed to x-rays for 8-12 hours using a 2 circle Diffractometer. A Stoe x-ray camera[2] records the diffraction pattern, and measurements of the arcs are made from

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[2] Stoe End. Cie GmBH Co., Rep. of Germany

the developed negative. The d-spacing is calculated using the Bragg equation, as follows:

$$d = \frac{n \lambda}{2 \sin \theta}$$

where n = order = 1

λ = wavelength of x-rays = 0.154 mm

θ = measured diffracted angle

The x-ray diffraction patterns were analyzed qualitatively on the basis of the strength and definition of the arcs and rings for α-keratin, amorphous keratin, and calcium salt. A numerical ranking was ascribed to the subjective analyses, to permit statistical comparisons. The categories used were as follows:

Description	Rank
absent	0
weak, cloudy, barely visible	1
medium to weak	2
medium	3
medium to strong	4
strong, distinct, easily visible	5

Elemental Analysis - Methods

Baleen samples were taken from the same location as those for x-ray diffraction analysis. For analysis of manganese, copper, zinc, boron and iron, the samples were oven-dried, and a 1.25 g sample of finely ground baleen was charred at 225°-275°C, then ashed at 450°C for at least 2 hours. The ash was dissolved in 2.5 ml of 2 N HCl, added to 10.0 ml of distilled H_2O, and filtered. For analysis of nitrogen, phosphorus, potassium, and magnesium, a 0.25 g oven-dried sample of ground baleen was boiled in 5 ml of concentrated H_2SO_4 for 1 hour. A small aliquot of 30% H_2O_2 was added to the cooled solution, before reheating it for 5 minutes. This latter procedure was repeated until the solution was clear. The final heating and digestion was for 20 minutes, before adding distilled H_2O to a volume of 250 ml. The solutions resulting from both the dry ashing and the wet digestion methods were analyzed by atomic absorption for potassium, magnesium, calcium, manganese, copper, zinc and iron, using a Varian A.A. 175[3] with acetylene flame for all elements except calcium, which was analyzed using nitrous oxide. Boron, phosphorous and nitrogen concentrations were determined colorimetrically using a multi-channel autoanalyzer (Autotechnicon II)[4].

A homogeneous preparation was made from an entire fin whale plate, and multiple subsamples were analyzed as controls with each set of samples from treated plates. In this way, we established the precision and reproduce-ability of each analysis, and enabling us to compare the results of control and treated samples with greater confidence.

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[3] Varian Techtron Ltd., Melbourne, Australia
[4] Technican Instruments Corp., Tarrytown, NY

Results

The analyses provided considerable information on the physical properties and structure of baleen. We noted that the method of preparation of the plates and the dimensions of the test pieces significantly affected the measurement of breaking strength and Young's modulus. Using a standardized approach and test template IV (Fig. 9.2:1), we determined that the breaking strength of fin and sei whale baleen (72.8 $\pm$ 12.1, n=7, and 90.4 $\pm$ 26.9, n=10) was significantly greater than that of the grey whale specimens (21.1 $\pm$ 5.9, n=8). Test pieces taken from different locations on the same plate showed wide variation in breaking strength, with no clear trend in the fin and sei plates. Gray whale baleen appeared to become progressively stronger with increasing distance from the gum.

Analysis of the tensiometric properties for each test piece showed a pattern which could be used as a basis for determining the effects of exposure to petroleum hydrocarbons. With increasing stress, the test piece lengthens at a constant and characteristic rate, until it reaches the point of yield, which is evident as a change in the slope of the curve (Figure 9.2:2 and 9.2:3). Thereafter, increased stress causes a disproportionate increase in length until the sample breaks.

For each test piece, we determined Young's modulus (stress vs strain over the initial portion of the curve), the stress at the point of yield, and the percent increase in length at the point of yield. Treatment with crude oil or gasoline had no effect on any of these parameters (Table 9.2:2). However, trichloroacetic acid significantly decreased the value of Young's modulus, aand increased the stress required to reach the point of yield. There was no change in the percent increase in length at the point of yield.

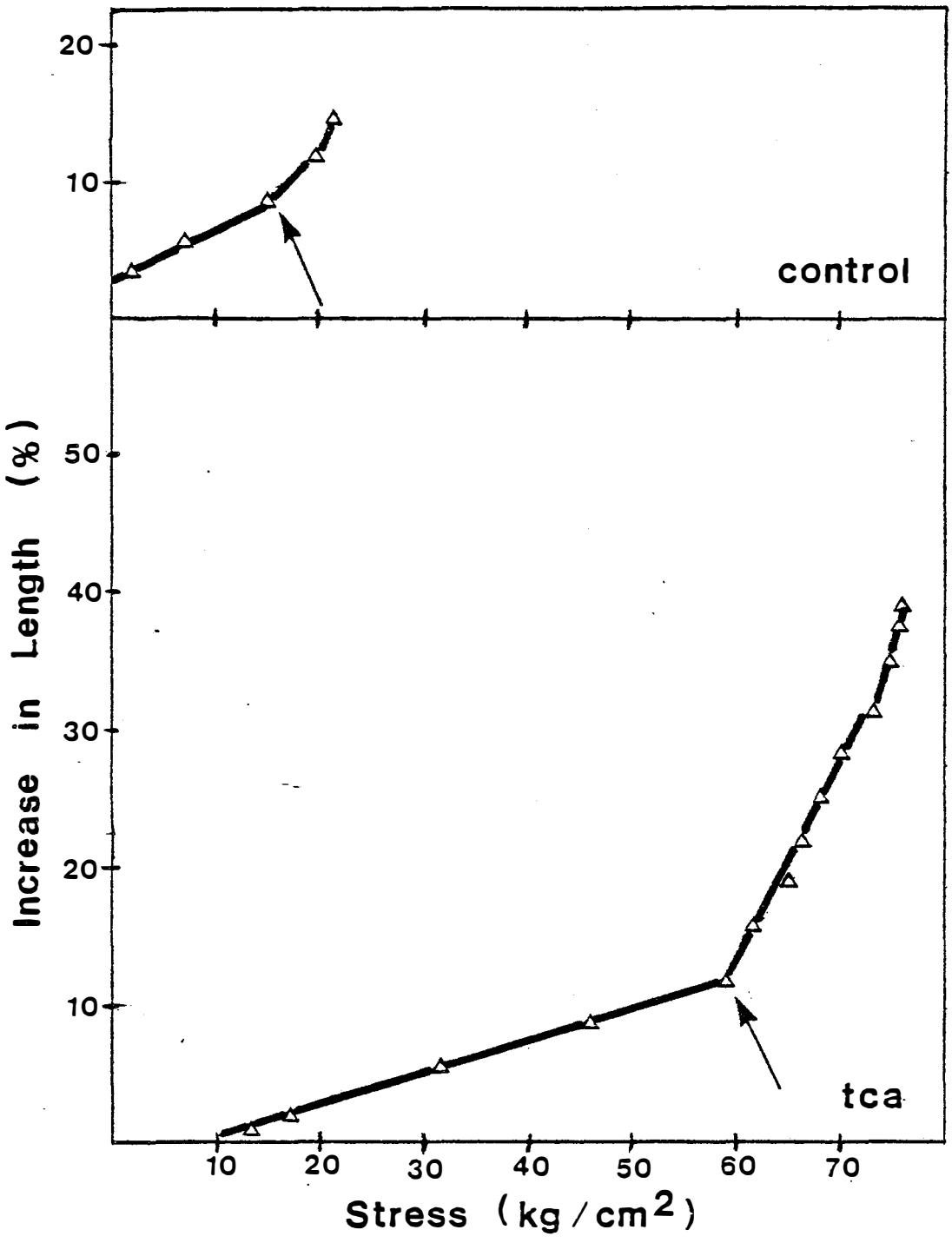

Figure 9.2:2 Representative stress curves for sections of fin whale baleen plates, taken 2.5-2.8 cm above the gum line. The point at which increased stress causes a disproportionate increase in length of the test piece is defined as the point of yield (arrow). Treatment of baleen with trichloracetic acid (TCA) causes a marked change in tensiometric properties.

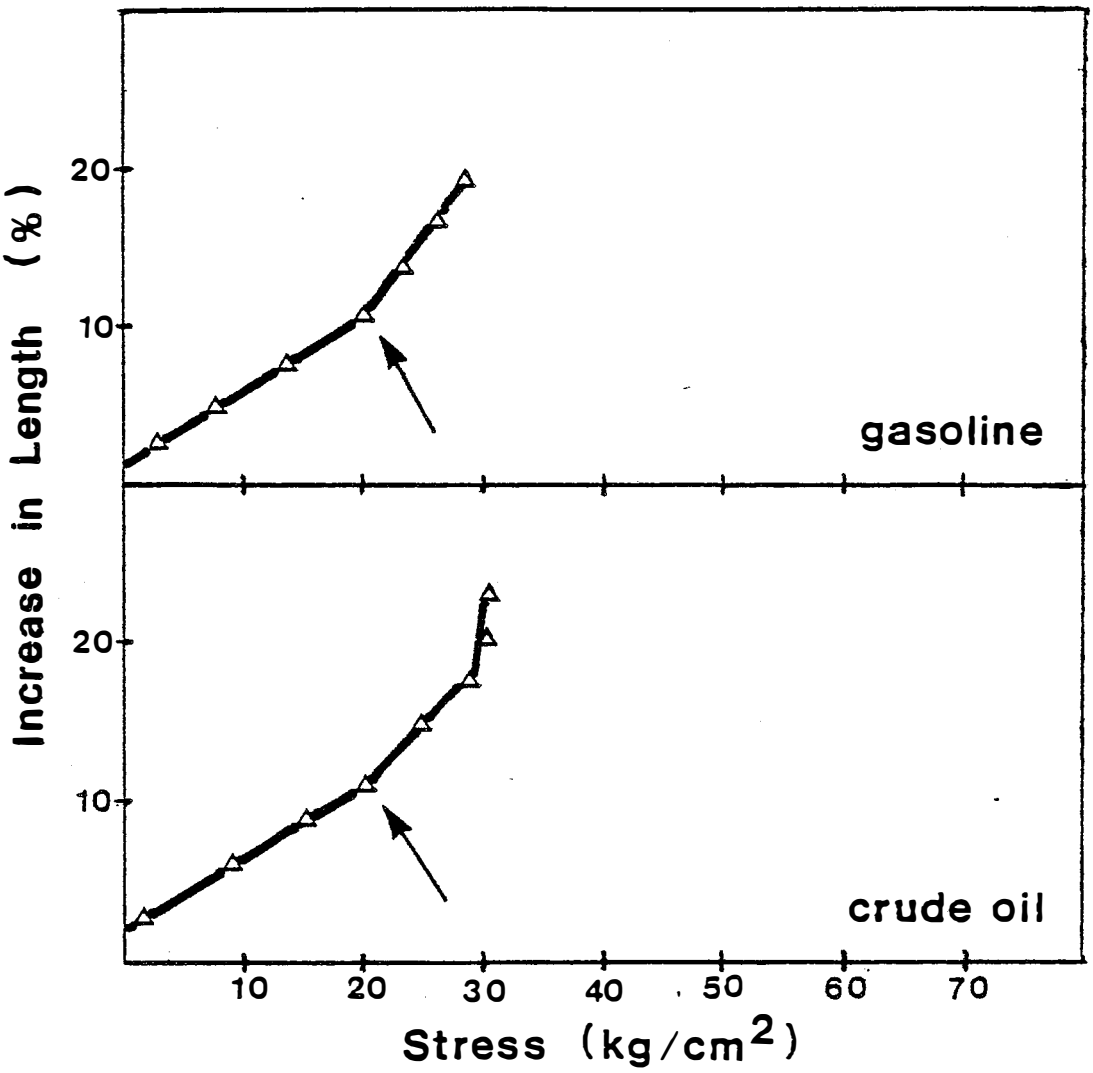

Figure 9.2:3 Representative stress curves for sections of fin whale baleen plates, taken 2.5-2.8 cm above the gum line. Exposure to gasoline or crude oil had no significant effect on the point of yield (arrow), when compared to control samples (see Figure 9.2:2).

Table 9.2:2 Tensile properties of fin whale baleen, treated with crude oil, lead-free gasoline, or decalcifying solution. Tests were carried out on pieces cut to the dimensions of template V (see Fig. 9.2:1).

Treatment	n	Young's Modulus ($N\ m^{-2} \times 10^{-7}$)	Percent Increase in length at Point of Yield	Stress at Point of Yield (kg/cm^2)	Breaking Strength (kg/cm^2)
control	7	1.72 ± 0.14	10.6 ± 1.6	20.4 ± 1.9	26.4 ± 1.8
crude oil	6	1.66 ± 0.13	9.8 ± 1.8	17.7 ± 2.2	26.0 ± 3.3
gasoline	6	1.64 ± 0.31	9.4 ± 2.4	16.9 ± 3.4	26.7 ± 1.7
t.c.a.	7	0.94 ± 0.14*	10.8 ± 2.7	52.1 ± 8.8	79.0 ± 11.1*

* $p < .001$

X-ray diffraction patterns revealed that the principle components of baleen are keratin and calcium salts; there was no evidence of collagen (Fig. 9.2:4). The keratin is either organized into an α-helix, or is present in an amorphous form. The calcium salts were principally hydroxyapatite and were most distinct in the fin, sei, humpback and minke whale samples. The gray and right whale samples had only faint evidence of calcium salt. The x-ray diffraction patterns for these species suggested that the crystals were quite small, and in one gray whale sample, perhaps represented a different salt, such as calcium oxalate. Our findings for the rorqual species are consistent with those of other studies (Pautard 1963); there are no published data for the gray whale. By contrast, we observed hydroxyapatite crystals in right whale samples, while other reports indicate that the baleen of the Greenaldn right whale, or bowhead, lacks calcium salts (Pautard 1963). Bowhead samples were not obtained in time to be examined for this report.

Comparison of the x-ray diffraction patterns from control and petroleum treated baleen plates suggested some changes in the keratin component of the matrix (Table 9.2:3). In the gray whale baleen, the α-keratin arcs were less readily detectable following all treatments, and the amorphous keratin was less distinct in tar-exposed plates. Tar improved the resolution of α-keratin arcs in diffraction patterns of right whale baleen, while fin whale baleen was unaffected by any petroleum treatment. As expected, decalcifying solution TCA) significantly decreased, and in many cases, eliminated the patterns for α- keratin and calcium salts, in all species. The effect of TCA on α-keratin was not anticipated, and suggests that calcium salts may play a role in stabilizing the keratin helix.

Elemental analysis of baleen plates and fibers revealed some interesting patterns. The nitrogen concentration of 7.5 to 11.5% indicates

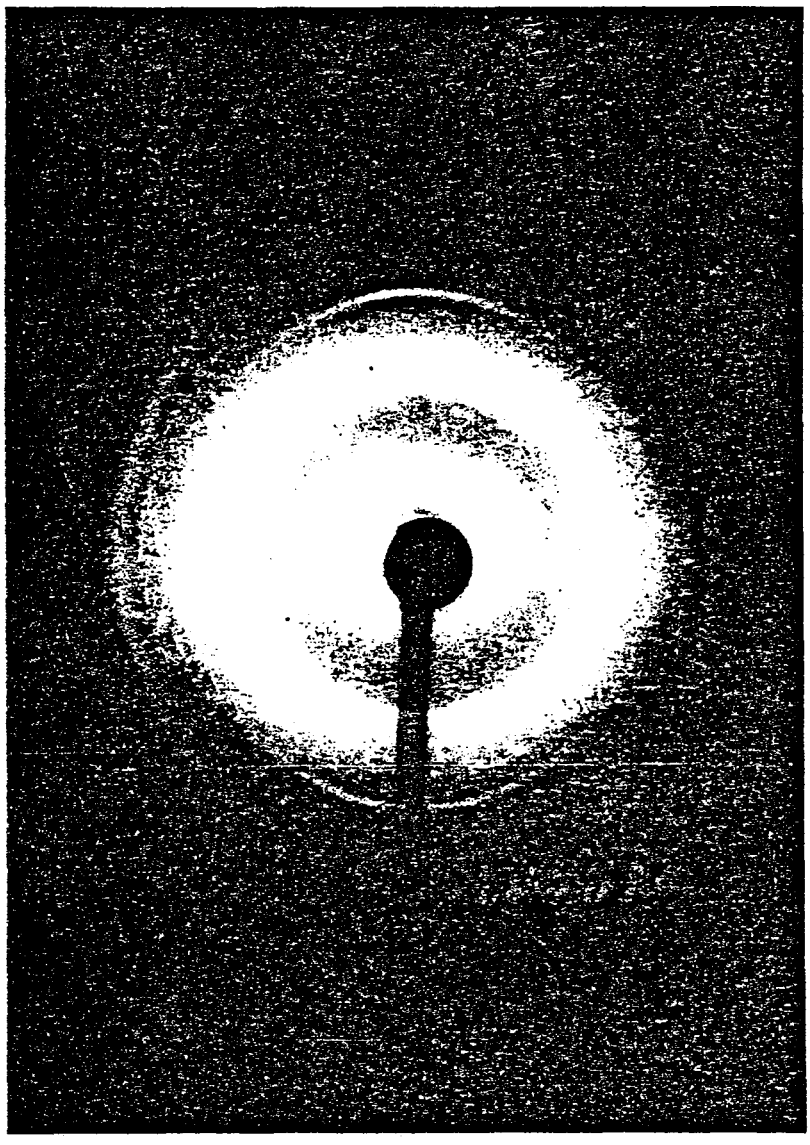

Figure 9.2:4 Low angle diffraction pattern from dry fin whale baleen.

Table 9.2:3 Characteristics of x-ray diffraction patterns of baleen from three mysticete species. Six samples were analyzed from each treated plate. See text for numeric classification of pattern strength and clarity.

		Characteristic		
Treatment	Duration	α-Keratin	Amorphous Keratin	Calcium Salts
FIN WHALE				
control[a]	-	3.0 ± 0.8	2.7 ± 1.4	1.7 ± 1.0
gasoline	1.5 h	2.0 ± 1.1	2.3 ± 0.8	1.2 ± 0.4
gasoline	14 d	3.3 ± 0.5	2.8 ± 0.4	2.2 ± 1.2
crude oil	8 h	2.3 ± 1.0	2.0 ± 1.1	2.3 ± 1.0
tar	21 d	3.0 ± 0.6	2.8 ± 0.4	2.7 ± 1.4
TCA	8 d	0.3 ± 0.5***	2.8 ± 0.4	0.5 ± 1.2*
RIGHT WHALE				
control	-	2.5 ± 1.0	2.7 ± 0.5	1 ± 0
gasoline	1.5 h	2.7 ± 0.5	2.2 ± 1.0	1 ± 0
crude oil	8 h	2.5 ± 0.8	2.2 ± 1.0	1 ± 0
tar	21 d	4.2 ± 0.4**	2.8 ± 0.4	1 ± 0
TCA	8 d	0***	2.7 ± 1.0	0[b]
GRAY WHALE				
control	-	4.3 ± 1.0	3.3 ± 1.0	1 ± 0
gasoline	1.5 h	2.2 ± 1.0**	2.5 ± 0.5	1 ± 0
crude oil	8 h	2.2 ± 0.8**	2.3 ± 0.5	1 ± 0
tar	21 d	2.2 ± 1.0**	1.7 ± 1.0*	1.2 ± 0.4
TCA	8 d	0***	2.7 ± 0.8	0[b]

[a] Six samples from each of two control plates were examined for fin whale. n = 12.

[b] Calculation of level of significance using t-test is not possible when both SD = 0.

* $p < .05$

** $p < .01$

*** $p < .001$

that protein comprises 47-70% of the dry weight. After all treatments, the nitrogen levels increased in both matrix and fibers (Table 9.2:4). Since the treatment solutions contained no detectable nitrogen, this apparent increase was likely the result of removal of endogenous lipid by solvent fractions in the petroleum, with a concurrent effect on the dry weight. Similar changes were not observed in the other elements tested, but none is present in as high concentrations as nitrogen, and the expected effect of decreased dry weight may have been masked by other factors.

Certain elements, notably manganese, copper, boron and iron, appear to be much more highly concentrated in the fibers than in the matrix of the plate (Table 9.2:5). It was these elements whch were most affected by exposure to petroleum. Though the sample size is small, there is a consistent decrease in these substances in both fin and gray whale fibers, but not in the plates. There was no such change in the right whale samples. The small differences in manganese and iron in the treated plates were not significant when evaluated against the precision of the analysis, which we established for each constituent.

In summary, prolonged exposure to petroleum substances does not seem to have any dramatic effects on the integrity of baleen plates. There is no tensiometric evidence to suggest increased fragility, and the keratinized matrix is qualitatively unaffected in most cases, as judged by x-ray diffraction patterns. The significance of the changes in α-keratin in gray whale baleen cannot be determined at this time. Exposure to petroleum causes some leaching of trace elements, particularly from the fibers, and may also remove lipids from both the matrix and the fibers. We did not determine how the loss of these components might affect baleen plates.

Table 9.2:4 Effects of petroleum exposure on the concentration of nitrogen in baleen from three species. One plate was analyzed for each treatment.

	NITROGEN CONCENTRATION (% dry weight)					
	FIN		GRAY		RIGHT	
Treatment	Plate[a]	Fibers[b]	Plate[a]	Fibers[b]	Plate[a]	Fibers[b]
Control	7.5(7.5-7.5)	10.0	10.7(10-12)	11.0	11.4(11-11.5)	6.6
Gasoline (90 min.)	11.9(11.5-12)	12.5	11.9(11.5-12)	13.5	11.6(11-12)	11.0
Gasoline (14 d)	13.4(13-13.5)	13.5	---	---	---	---
Crude Oil (8 h)	13.5(13-14)	13.0	13.6(13-14.5)	13.5	13.6(13.5-14)	7.8
Tar (21 d)	13.1(13-13.5)	12.0	13.0(12-13.5)	13.5	13.1(12.5-13.5)	12.0

[a] $n = 4$, $\bar{x}$ (range).
[b] $n = 1$

Table 9.2:5 Effects of petroleum exposure on the concentration of Mn, Cu, B, and Fe in fin and gray whale baleen. One plate was analyzed for each treatment.

	ELEMENTAL CONTENT (ppm)							
	Manganese		Copper		Boron		Iron	
Treatment	Plate[a]	Fibers[b]	Plate[a]	Fibers[b]	Plate[a]	Fibers[b]	Plate[a]	Fibers[b]
FIN WHALE								
Control	0.2(0-1)	10	18(17-21)	34	5.0(3-7)	10	12(7-23)	300
Gasoline (90 min.)	0.7(0-1)	2	12(10-13)	19	5.2(3-8)	3	27(11-58)	29
Gasoline (14 days)	1.0(1-1)	6	14(14-14)	19	5.2(4-7)	5	5(0-11)	18
Crude Oil (8 hrs.)	n.d.	3	13(12-13)	15	4.2(2-7)	4	20(15-23)	128
Tar (21 days)	1.0(1-1)	6	14(13-16)	14	5.2(3-8)	7	6(4-7)	86
GRAY WHALE								
Control	1.5(1-2)	26	6(4-10)	16	3.7(2-5)	9	37(7-59)	543
Gasoline (90 min.)	0.7(0-1)	2	7(6-7)	9	2.5(1-5)	3	27(8-76)	95
Crude Oil (8 hrs.)	0.5(0-1)	4	6(6-7)	7	3.2(2-4)	4	61(15-83)	295
Tar (21 days)	0.5(0-1)	3	6(5-6)	7	2.5(2-3)	4	4(3-6)	22

a - n = 4 subsamples from each plate
b - n = 1 sample of fibers

References

(1) Anonymous. 1973. Annual Book of ASTM Standards. Part 27. Plastics - General Methods of Testing Nomenclature. American Society for Testing and Materials, Philadelphia, PA 19103.

(2) LaBarbera, M., and S. Vogel. 1976. An inexpensive thermistor flowmeter for aquatic biology. Limnology and Oceanography 21: 750-756.

(3) Pautard, F.J.E. 1963. Mineralization of keratin and its comparison with the enamel matrix. Nature 199: 531-535.

(4) Pivorunas, A. 1979. The feeding mechanisms of baleen whales. Am. Scientist 67 (4): 432-440.

10.00 OIL SPILL RESPONSE

The IXTOC II wellhead in the Gulf of Campeche was the site of great activity during the fall and winter of 1979-80. There were occasional unconfirmed reports of marine mammals either in oil, or associated with oil from the blowout at the wellhead.

On October 15, 1979, we established contact with Dr. T. Fritts of the U.S. Fish and Wildlife Service, National Fish and Wildlife Laboratory, Belle Chasse, Louisiana. Dr. Fritts was conducting aerial surveys out to the area of the continental shelf off Florida, Louisiana and Texas. The U.S. Coast Guard was conducting concurrent flights from St. Petersburg, Florida, to the wellhead in order to record oil dispersion patterns.

Neither agency was able to conduct complete and detailed surveys over the spill area. On the basis of the surveys that were conducted, there did not appear to be sufficient justification to dispatch an Oil Spill Response Team to the site.

Dr. Geraci prepared to accompany a U.S. Coast Guard survey on March 18, 1980. That week marked the end of the blowout, and the termination of Coast Guard aerial surveys over IXTOC II.

Under BLM support, the Fish and Wildlife Service began a new survey program, with flights originating from Brownsville, Texas, Marsh Island, Louisiana, Naples, Florida, and Cape Canaveral, Florida. D. St. Aubin accompanied the FWS survey team on two flights originating from Brownsville, Texas, on August 20 and 21, 1980. The survey team noted substantial fouling of the water and beaches north of Tampeco, Mexico, within the coastal waters of Texas. Marine mammals, including sperm whales, beaked whales and various delphinids were observed, but not associated with oil.

On May 6, 1981, two cargo ships collided off Nags Head, North Carolina, resulting in a minor oil spill. More than 3000 gallons of #4 fuel oil reached the beaches of the Outer Banks by May 8. The Associated Press reported that a porpoise and an undetermined number of birds had been killed. However, biologists contacted at the Pea Island National Wildlife Refuge and the North Carolina Marine Resources Department denied the report that the "porpoise" had been killed by oil. This was confirmed by field personnel from the Smithsonian Institution, who carried out a 50-mile beach survey. Eight cetacean carcasses were recovered, including two Grampus griseus, two Tursiops truncatus, one Kogia breviceps, a Phocoena phocoena, a Stenella sp., and a Globicephala sp. Only four of the specimens were fresh enough to have died within the time frame of the oil spill. These included a 105 cm neonatal Tursiops, which appeared to have died as a result of separation or abandonment by its mother, a Stenella sp. with extensive rope marks around its tail suggesting that it had been entangled, a Kogia breviceps, and a Grampus griseus. None of these had any surface evidence of oil. The circumstances did not warrant any further activity or mobilization of the Oil Spill Response Team.