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*Vet Pathol* 1993 30: 1
DOI: 10.1177/030098589303000101

Vet Pathol 30:1-11 (1993)

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Abstract. Following the *Exxon Valdez* oil spill in Prince William Sound, Alaska, sea otters *(Enhydra lutris)* that appeared to be contaminated with oil, that were in danger of becoming contaminated, or that were behaving abnormally were captured and taken to rehabilitation centers. Exposure to oil was assessed by visual examination when otters arrived at the centers. Degree of oil exposure was graded according to the following criteria: oil covering greater than 60% of the body-heavily contaminated; oil covering 30-60% of the body-moderately contaminated; oil covering less than 30% of the body or light sheen on fur- lightly contaminated. If there was no oil visible, otters were considered uncontaminated. Tissues from 51 oil-contaminated sea otters (14 males, 37 females) and from six uncontaminated sea otters (three males, three females) that died in rehabilitation centers were examined histologically. Among oil-contaminated sea otters, 19/46 had interstitial pulmonary emphysema, 13/40 had gastric erosion and hemorrhage, 11/47 had centrilobular hepatic necrosis, 14/47 had periportal to diffuse hepatic lipidosis, and 10/42 had renal tubular lipidosis. Of the uncontaminated sea otters, 1/6 had gastric erosion and hemorrhage and 1/6 had diffuse hepatic lipidosis. Histologic examinations were performed on tissues from five sea otters (three males, two females) found dead with external oil present 15 to 16 days after the spill. Periportal hepatic lipidosis and renal tubular lipidosis were found in 3/5, and interstitial pulmonary emphysema was found in 1/5. Tissues from six apparently normal sea otters (four males, two females) collected from an area not affected by an oil spill were examined histologically, and none of these lesions were found. We conclude that interstitial pulmonary emphysema, centrilobular hepatic necrosis, and hepatic and renal lipidosis of sea otters were associated with exposure to crude oil. Gastric erosion and hemorrhage may have been associated with stress of captivity and/or oil exposure.

*Key words:* Crude oil; *Exxon Valdez* oil spill; interstitial pulmonary emphysema; petroleum hydrocarbon; sea otters.

On 24 March 1989, the oil tanker *Exxon Valdez* ran aground on Bligh Reef in Prince William Sound, Alaska. The resulting spill of approximately 41.6 million liters of North Slope crude oil was the largest in the history of the United States. In the months following the spill, over 1,000 sea otters *(Enhydra lutris)* from oil spill-affected areas are known to have died. The actual number that died was probably much greater. The purpose of this study is to identify and describe histopathologic lesions associated with crude oil exposure in sea otters and to discuss possible pathogeneses of the lesions. Materials available included tissues from oil-contaminated and uncontaminated otters that died in rehabilitation centers following the oil spill and tissues from otters that were found dead in the oil spill-affected area with external oil present. Tissues from apparently normal sea otters from an area not contaminated by crude oil, were also examined.

## Materials and Methods

Following the oil spill, sea otters *(Enhydra lutris)* that appeared oil contaminated, that were in danger of becoming oil contaminated, or that were behaving abnormally were captured and taken to rehabilitation centers. Oil exposure was assessed by visual examination on arrival at the centers. Degree of oil contamination was graded according to the following criteria: oil covering greater than 60% of the body-heavily contaminated; oil covering 30-60% of the body-moderately contaminated; oil covering less than 30% of the body or light sheen on fur-lightly contaminated. If there was no oil visible, otters were considered uncontaminated.

Fifty-one oil-contaminated otters (14 males and 37 females) died in rehabilitation centers (Group No. 1). Six uncontaminated otters (three males and three females) died in rehabilitation centers (Group No. 2). Five otters (three males and two females) were found dead with external oil present (Group No. 3). Six apparently healthy sea otters (four males and two females) were killed by gunshot in an area not af-

**Table 1.** Sex, arrival date, death date, number of days at rehabilitation center, and lesions found in 1) heavily, moderately, and lightly oil-contaminated and 2) uncontaminated sea otters that died in rehabilitation centers, and found in 3) oil-contaminated sea otters that were found dead, and in 4) uncontaminated sea otters that were killed in the wild.

| Group Number (characteristics) | Otter Number | Sex[1] | Arrival Date (1989) | Death Date (1989) | Days at Center | Lesions[2] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | EMP | GE | HL | RL | CLHN |
| I (oil-contaminated, died in center) | | | | | | | | | | |
| Heavily oil contaminated | 1 | F | 7 April | 7 April | < 1 | X‡ | X | X | O§ | 0 |
| | 2 | F | 4 April | 5 April | 1 | 0 | X | 0 | 0 | 0 |
| | 3 | F | 9 April | 10 April | 1 | X | 0 | X | X | 0 |
| | 4 | F | 6 April | 7 April | 1 | X | 0 | X | 0 | 0 |
| | 5 | F | 6 April | 7 April | 1 | 0 | 0 | 0 | 0 | X |
| | 6 | F | 5 April | 8 April | 3 | X | 0 | X | X | 0 |
| | 7 | F | 31 March | 3 April | 3 | X | 0 | X | 0 | 0 |
| | 8 | F | 4 April | 7 April | 3 | X | 0 | 0 | 0 | 0 |
| | 9 | F | 19 April | 23 April | 4 | 0 | 0 | X | X | 0 |
| | 10 | F | 3 April | 7 April | 4 | X | 0 | 0 | 0 | 0 |
| | 11 | F | 5 April | 10 April | 5 | X | 0 | X | X | 0 |
| | 12 | M | 30 March | 5 April | 6 | X | 0 | 0 | 0 | 0 |
| | 13 | M | 2 April | 9 April | 7 | X | 0 | 0 | 0 | X |
| | 14 | F | 1 April | 9 April | 8 | X | 0 | X | X | X |
| | 15 | M | 1 April | 10 April | 9 | 0 | 0 | 0 | 0 | 0 |
| | 16 | M | 2 April | 28 July | 117 | 0 | 0 | 0 | 0 | X |
| Moderately oil contaminated | 17 | F | 9 April | 10 April | 1 | 0 | 0 | X | X | 0 |
| | 18 | F | 4 April | 5 April | 1 | X | 0 | X | X | X |
| | 19 | F | 8 April | 9 April | 1 | X | X | X | X | 0 |
| | 20 | F | 7 April | 8 April | 1 | 0 | 0 | X | X | 0 |
| | 21 | F | 6 April | 8 April | 2 | X | 0 | X | X | X |
| | 22 | F | 3 April | 6 April | 3 | X | X | 0 | 0 | 0 |
| | 23 | M | 9 April | 13 April | 4 | 0 | X | 0 | 0 | 0 |
| | 24 | F | 4 April | 9 April | 5 | 0 | X | 0 | 0 | X |
| | 25 | M | 18 April | 29 April | 11 | 0 | 0 | 0 | 0 | 0 |
| | 26 | F | 5 April | 18 April | 13 | X | 0 | 0 | 0 | 0 |
| | 27 | F | 11 May | 24 May | 13 | 0 | X | 0 | 0 | 0 |
| | 28 | M | 5 April | 5 May | 30 | 0 | X | 0 | 0 | 0 |
| Lightly oil contaminated | 29 | F | 11 May | 24 July | 74 | 0 | X | 0 | 0 | 0 |
| | 30 | F | 20 April | 20 April | < 1 | 0 | 0 | 0 | 0 | X |
| | 31 | F | 5 June | 5 June | < 1 | 0 | 0 | 0 | 0 | 0 |
| | 32 | F | 6 April | 7 April | 1 | 0 | 0 | 0 | 0 | 0 |
| | 33 | F | 5 June | 6 June | 1 | 0 | 0 | 0 | 0 | 0 |
| | 34 | M | 13 June | 14 June | 1 | 0 | X | 0 | 0 | 0 |
| | 35 | F | 9 April | 11 April | 2 | 0 | 0 | X | 0 | 0 |
| | 36 | F | 1 April | 4 April | 3 | 0 | 0 | 0 | 0 | 0 |
| | 37 | F | 4 April | 7 April | 3 | X | X | 0 | 0 | 0 |
| | 38 | M | 6 April | 12 April | 6 | X | 0 | 0 | 0 | 0 |
| | 39 | F | 10 May | 17 May | 7 | 0 | 0 | 0 | 0 | 0 |
| | 40 | M | 19 April | 27 May | 8 | 0 | 0 | 0 | 0 | X |
| | 41 | F | 25 May | 4 June | 10 | 0 | X | 0 | 0 | 0 |
| | 42 | M | 20 May | 31 May | 11 | 0 | 0 | 0 | 0 | 0 |
| | 43 | F | 13 June | 27 June | 14 | 0 | 0 | 0 | 0 | 0 |
| | 44 | F | 8 April | 28 April | 20 | 0 | X | 0 | 0 | X |
| | 45 | M | 8 April | 20 April | 21 | 0 | 0 | 0 | 0 | 0 |
| | 46 | M | 8 April | 1 May | 23 | 0 | 0 | 0 | 0 | 0 |
| | 47 | M | 6 April | 29 April | 23 | 0 | 0 | 0 | 0 | 0 |
| | 48 | F | 6 April | 30 April | 24 | X | 0 | 0 | 0 | 0 |
| | 49 | F | 10 April | 6 May | 26 | 0 | 0 | 0 | 0 | X |
| | 50 | F | 11 May | 7 June | 27 | 0 | 0 | 0 | 0 | 0 |
| | 51 | F | 20 May | 19 June | 30 | 0 | 0 | 0 | 0 | 0 |

Table 1. Continued.

| Group Number (Characteristics) | Otter Number | Sex* | Arrival Date (1989) | Death Date (1989) | Days at Center | Lesions† | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | EMP | GE | HL | RL | CLHN |
| 2 (uncontaminated, died in center) | 52 | F | 29 May | 29 May | < 1 | 0 | 0 | 0 | 0 | 0 |
| | 53 | M | 13 April | 14 April | 1 | 0 | 0 | 0 | 0 | 0 |
| | 54 | F | 5 July | 6 July | 1 | 0 | 0 | X | 0 | 0 |
| | 55 | M | 25 June | 27 June | 2 | 0 | 0 | 0 | 0 | 0 |
| | 56 | F | 19 June | 3 July | 14 | 0 | 0 | 0 | 0 | 0 |
| | 57 | M | 17 July | 4 August | 18 | 0 | X | 0 | 0 | 0 |
| 3 (oil contaminated, found dead in wild) | 58 | M | NA‖ | NA | NA | 0 | 0 | 0 | 0 | 0 |
| | 59 | M | NA | NA | NA | 0 | 0 | X | X | 0 |
| | 60 | M | NA | NA | NA | 0 | 0 | 0 | 0 | 0 |
| | 61 | F | NA | NA | NA | 0 | 0 | X | X | 0 |
| | 62 | F | NA | NA | NA | X | 0 | X | X | 0 |
| 4 (uncontaminated, killed in wild) | 63 | F | NA | NA | NA | 0 | 0 | 0 | 0 | 0 |
| | 64 | F | NA | NA | NA | 0 | 0 | 0 | 0 | 0 |
| | 65 | M | NA | NA | NA | 0 | 0 | 0 | 0 | 0 |
| | 66 | M | NA | NA | NA | 0 | 0 | 0 | 0 | 0 |
| | 67 | M | NA | NA | NA | 0 | 0 | 0 | 0 | 0 |
| | 68 | M | NA | NA | NA | 0 | 0 | 0 | 0 | 0 |

* F = female; M = male.
† EMP = interstitial pulmonary emphysema; GE = gastric erosion; HL = hepatic lipidosis; RL = renal lipidosis; CLHN = centrilobular hepatic necrosis.
‡ X = lesion present.
§ 0 = lesion absent.
‖ NA = not applicable.

fected by an oil spill as part of unrelated research (Group No. 4).

The sea otters included in this study were necropsied by various individuals. This fact and the lack of a standard necropsy protocol during the first few weeks after the spill resulted in variation in the tissues collected. Similarly, specimens for toxicologic analysis for petroleum hydrocarbons frequently were not collected or were collected improperly. Thus, tissue petroleum hydrocarbon analyses are not included in this study. Documentation of necropsy findings ranged from minimal to thorough. In some cases, no necropsy report was available. Only otters with documented oil exposure assessment were included in this study. Otters that died in rehabilitation centers had been collected from oil-contaminated areas of Prince William Sound from 30 March to 17 July 1989 and had died between 3 April and 4 August 1989. Animals that were found dead and necropsied had been collected from contaminated areas on 8 and 9 April 1989. The six apparently healthy sea otters (Group No. 4) were collected during the summer of 1989 from the waters surrounding the Kuril Islands, Union of Soviet Socialist Republics. Although these otters were collected from a different area at a different time of year, they were the best control animals available. Pups were not included in the study because of the small number available.

The following tissues were fixed in 10% neutral buffered formalin, dehydrated, and processed in paraffin: adrenal gland, aorta, bone marrow, brain, esophagus, eye, heart, intestine, kidney, liver, lung, lymph node, mammary gland, ovary, pancreas, parathyroid, pituitary gland, skeletal muscle, skin, spinal cord, spleen, stomach, testis, thymus, thyroid, tongue, tonsil, trachea, urinary bladder, and uterus. Not all of these were collected from each otter. In some cases, tissues in which lesions were commonly found (lung, stomach, liver, and kidney) were not collected. Sections were cut at 5 $\mu$m and stained with hematoxylin and eosin. Selected sections were stained with oil red O.

## Results

Data on individual otters are presented in Table 1. Numbers of otters of each sex with each of the common lesions are presented in Table 2.

In oil-contaminated sea otters that died in rehabilitation centers (Group No. I), interstitial pulmonary emphysema was the most prevalent lesion: 11/15 (73%) heavily contaminated, 5/11 (45%) moderately contaminated, and 3/20 (15%) lightly contaminated otters. Overall, the lesion was present in 19/46 (41%) otters. It was common in heavily and moderately contaminated Group No. I otters that died within 8 days of arrival at the rehabilitation centers: 16/22 (73%) animals. Histologically, the lesion appeared as expanded areas of clear space with rounded contours within the interlobular septa (Fig. I). Occasionally, adjacent parenchyma was compressed or atelectatic.

Gastric erosions, here defined as focal areas of gastric mucosal necrosis with or without sloughing of the necrotic tissue, were seen in 2/14 (14%) heavily contam-

Table 2. Oil contamination status, sex, and lesions found in 1) oil-contaminated and 2) uncontaminated sea otters that died in rehabilitation centers, in 3) oil-contaminated sea otters found dead, and in 4) uncontaminated sea otters killed in the wild.

| Group No. (Characteristics) | Oil Contamination Status* | Sex† | n | Emphysema | | Gastric Erosion | | Hepatic Lipidosis | | Renal Lipidosis | | Centrilobular Necrosis | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | No. Affected/ Total Examined‡ | % | No. Affected/ Total Examined‡ | % | No. Affected/ Total Examined‡ | % | No. Affected/ Total Examined‡ | % | No. Affected/ Total Examined‡ | % |
| 1 (oil contaminated, died in center) | HC | F | 12 | 9/11 | 82 | 2/10 | 20 | 8/12 | 67 | 5/11 | 45 | 2/12 | 17 |
| | HC | M | 4 | 2/4 | 50 | 0/4 | 0 | 0/4 | 0 | 0/2 | 0 | 2/4 | 50 |
| | HC | F, M | 16 | 11/15 | 73 | 2/14 | 14 | 8/16 | 50 | 5/13 | 38 | 4/16 | 25 |
| | MC | F | 10 | 5/8 | 62 | 5/6 | 83 | 5/9 | 56 | 5/9 | 56 | 3/9 | 33 |
| | MC | M | 3 | 0/3 | 0 | 2/3 | 67 | 0/3 | 0 | 0/3 | 0 | 0/3 | 0 |
| | MC | F, M | 13 | 5/11 | 45 | 7/9 | 78 | 5/12 | 42 | 5/12 | 42 | 3/12 | 25 |
| | LC | F | 15 | 2/14 | 14 | 3/13 | 23 | 1/12 | 8 | 0/12 | 0 | 3/12 | 25 |
| | LC | M | 7 | 1/6 | 17 | 1/4 | 25 | 0/7 | 0 | 0/5 | 0 | 1/7 | 14 |
| | LC | F, M | 22 | 3/20 | 15 | 4/17 | 24 | 1/19 | 5 | 0/17 | 0 | 4/19 | 21 |
| | TC | F | 37 | 16/33 | 48 | 10/29 | 34 | 14/33 | 42 | 10/32 | 31 | 8/33 | 24 |
| | TC | M | 14 | 3/13 | 23 | 3/11 | 27 | 0/14 | 0 | 0/10 | 0 | 3/14 | 21 |
| | TC | F, M | 51 | 19/46 | 41 | 13/40 | 32 | 14/47 | 30 | 10/42 | 24 | 11/47 | 23 |
| 2 (uncontaminated, died in center) | U | F | 3 | 0/3 | 0 | 0/3 | 0 | 1/3 | 33 | 0/3 | 0 | 0/3 | 0 |
| | U | M | 3 | 0/3 | 0 | 1/3 | 33 | 0/3 | 0 | 0/3 | 0 | 0/3 | 0 |
| | U | F, M | 6 | 0/6 | 0 | 1/6 | 17 | 1/6 | 17 | 0/6 | 0 | 0/6 | 0 |
| 3 (oil contaminated, found dead) | TC | F | 2 | 1/2 | 50 | 0/12 | 0 | 2/12 | 100 | 2/12 | 100 | 0/12 | 0 |
| | TC | M | 3 | 0/3 | 0 | 0/3 | 0 | 1/3 | 33 | 1/3 | 33 | 0/3 | 0 |
| | TC | F, M | 5 | 1/5 | 20 | 0/5 | 0 | 3/5 | 60 | 3/5 | 60 | 0/5 | 0 |
| 4 (uncontaminated, killed in wild) | U | F, M | 6 | 0/0 | 0 | 0/0 | 0 | 0/0 | 0 | 0/0 | 0 | 0/0 | 0 |

* HC = heavily oil contaminated; MC = moderately oil contaminated; LC = lightly oil contaminated; TC = total oil contaminated; U = uncontaminated.

† F = female; M = male,

‡ Denominator does not always equal n because some tissues were not available for some otters.

inated, 7/9 (78%) moderately contaminated, and 4/17 (24%) lightly contaminated Group No. I sea otters. Among total Group NO. 1 otters, 13/40 (32%) had the lesion. Gastric erosions were common in otters that died during the first 5 days in the centers (eight animals) and also occurred in animals that died after 10, 13, 20, 30, and 74 days. Histologically, the erosions appeared as discrete areas of coagulative necrosis 1-3 mm in diameter and affected superficial to midlevel gastric mucosa (Fig. 2). Variable amounts of hemorrhage and dark brown pigment produced by acid digestion of blood were present in the necrotic areas. Small numbers of neutrophils were sometimes scattered along the margins of the erosions. In some cases, the necrotic tissue sloughed into the gastric lumen, leaving sharply demarcated mucosal defects. Gastric ulcers, which by definition penetrate the muscularis mucosa, were not seen.

Hepatic lipidosis was present in 8/16 (50%) heavily contaminated, 5/12 (42%) moderately contaminated, and 1/19 (5%) lightly contaminated Group No. I otters. Among total Group No.1 otters, 14/47 (30%) had the lesion. The sex ratio of Group No. 1 otters was 1 male:2.6 females. All Group No. I animals with hepatic lipidosis were female. Three of the affected animals were pregnant, and 11 were not. The lesion was common in heavily and moderately contaminated otters that died within 8 days of arrival at the centers (13/22 [59%]) and was not found in animals that died later. The lesion was characterized by the presence of variably sized, usually multiple but occasionally single, round, sharply delineated, unstained intracytoplasmic vacuoles in periportal hepatocytes; in the more severely affected livers, midzonal and centrilobular hepatocytes also contained vacuoles (Fig. 3). In oil red O-stained sections, the intracytoplasmic vacuoles stained red, indicating the presence of lipid.

The prevalence of renal lipidosis was somewhat less than that of hepatic lipidosis: 10/42 (24%) Group No. I otters. All otters with renal lipidosis were female and

also had hepatic lipidosis. Two otters with renal lipidosis were pregnant, and eight were not. As with hepatic lipidosis, renal lipidosis was common in heavily and moderately contaminated otters that died within 8 days of arrival at the centers: 10/22 (45%). Microscopically, affected kidneys had single or multiple, variably sized, round, discrete, unstained intracytoplasmic vacuoles within proximal and distal tubular epithelium (Fig. 4). The vacuoles stained red with oil red O. In one kidney with lipidosis, a few tubules contained irregularly shaped, basophilic, isotropic crystalline structures that were not identified.

Centrilobular hepatic necrosis occurred in 4/16 (25%) heavily contaminated, 3/12 (25%) moderately contaminated, and 4/19 (21%) lightly contaminated Group No. I otters. Among all Group No. I otters, 11/47 (23%) were affected. The lesion was seen in otters that died on the day of arrival and after 1, 2, 5, 7, 8, 20, 26, and 117 days in the centers. In affected livers, centrilobular hepatocytes had undergone coagulative necrosis characterized by pyknosis, karyorrhexis, karyolysis, and increased eosinophilia of cytoplasm with preservation of basic cell shape (Fig. 5). Among all Group No. 1 otters, multifocal hepatic necrosis was present in 6/47 (13%), and the presence of focally extensive hepatic necrosis suggested infarction in 4/47 (8%). Multifocal hepatic necrosis appeared as randomly distributed areas of either lytic or coagulative necrosis of small groups of hepatocytes accompanied by few inflammatory cells. No cause for the necrosis was found. Multifocal hepatic necrosis occurred in one otter that died on the first day of captivity and in otters that died after 3, 4, 5, 26, and 27 days. Focally extensive hepatic necrosis consisted of sharply demarcated areas of coagulative necrosis that included several contiguous hepatic lobules or large regions of hepatic lobes. No vascular lesions or other causes were evident. This pattern was found in animals that died after 4, 6, 8, and 27 days.

Of the six uncontaminated otters that died in rehabilitation centers (Group No.2), one male (17%) had gastric erosions, one female (17%) had periportal hepatic lipidosis and multifocal hepatic necrosis, and one female (17%) had focally extensive hepatic necrosis. The remaining Group No. 2 otters included one with peritonitis caused by a small intestinal perforation associated with severe acanthocephalan infection. Another had mild acute enteritis and mild subacute hepatitis of inapparent cause. The last otter in this group had no significant histologic lesions, and no necropsy report was available.

Of the five sea otters found dead with external oil present (Group No.3), one female had interstitial pulmonary emphysema, periportal hepatic lipidosis, and renal tubular lipidosis, and two others (one male and one female) had similar hepatic and renal lipidosis.

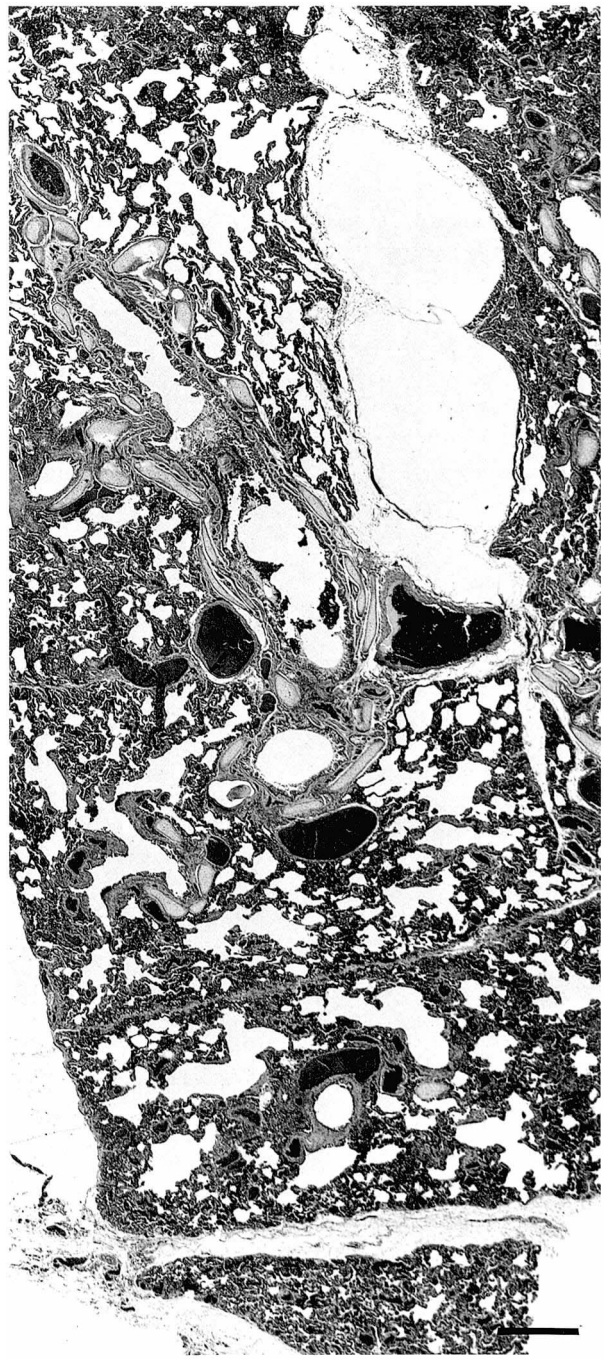

Fig. 1. Lung; otter No. II, interstitial emphysema. Interlobular septum is expanded by gas bubbles. Adjacent alveoli are atelectatic. HE. Bar = 200 μm.

The remaining two otters in this group were both male; no significant lesions were found, and no necropsy reports were available.

The six apparently healthy sea otters collected from an area that had not been affected by an oil spill (Group No.4) did not have interstitial pulmonary emphysema, gastric erosions, hepatic or renal lipidosis, hepatic necrosis, or other significant lesions. Four were male and two were nonpregnant females.

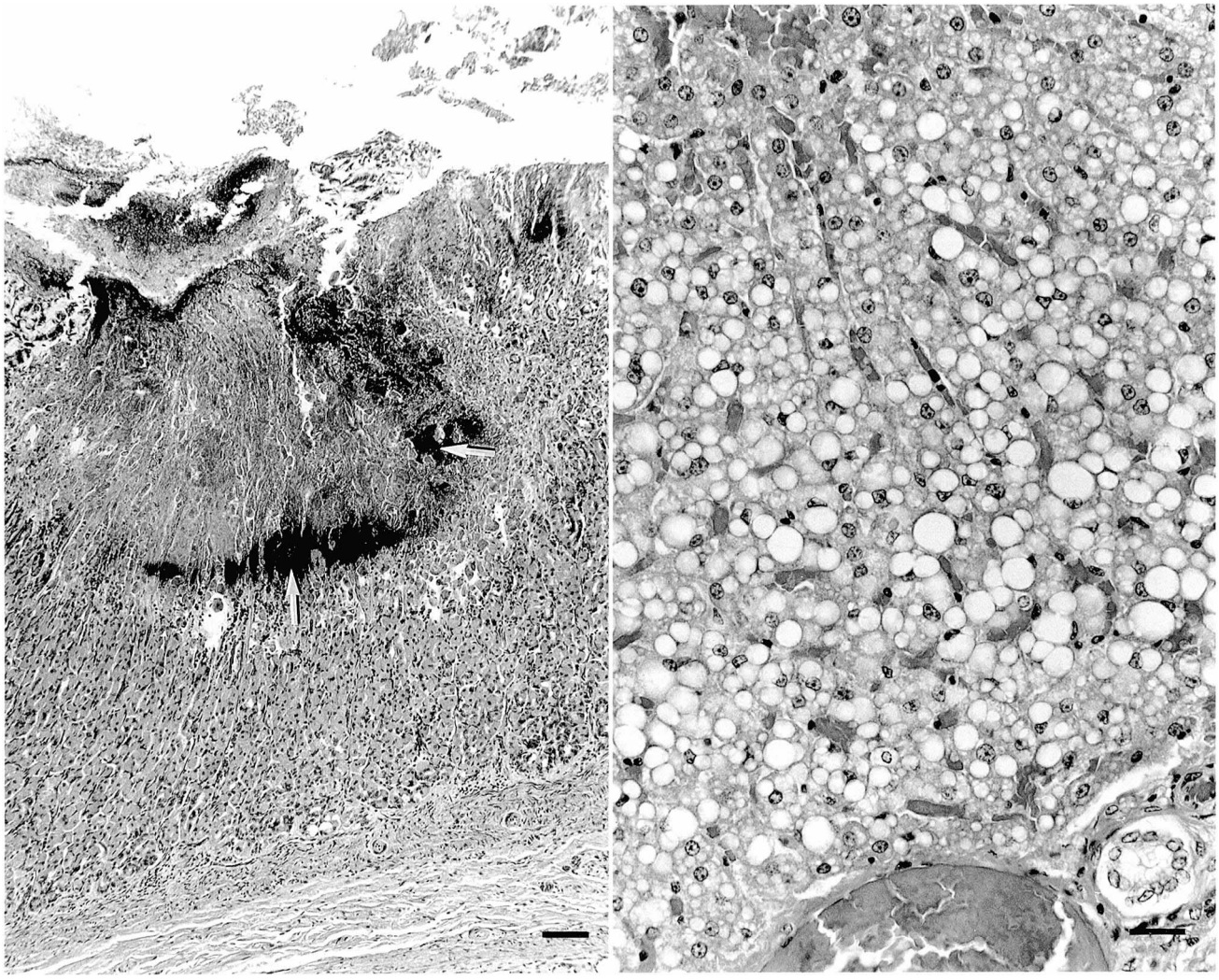

**Fig.** 2. Gastric mucosa; otter No. 22, focal area of coagulative necrosis. Note dark pigment produced by acid digestion of blood (arrows). HE. Bar = 100 μm.

Fig. 3. Liver; otter No. 20, diffuse lipidosis that is more severe in periportal hepatocytes. Central vein is at the top left. HE. Bar = 25 μm.

Various incidental lesions were found in animals in all groups. Thyroid follicular ectasia was common in animals in all groups.

## Discussion

A number of limitations are inherent in this study. The duration of oil exposure of individual otters could not be determined. The lack of necropsy reports for some otters and of sufficiently detailed necropsy reports for others impaired the ability to determine cause of death in some cases. The failure to collect or to properly collect specimens for determination of tissue petroleum hydrocarbon levels was also unfortunate. These deficiencies were caused in large measure by the remoteness of the area affected by the oil spill and the absence of adequate contingency plans and protocols for responding to such an event. Satisfactory necropsy and toxicology protocols were eventually put in use, but very few of the otters in this study died during that time. Nevertheless, all otters included in this study have documented oil exposure assessment. Many of the otters in this study were being held in captivity when they died. This is problematic because mortality resulting from factors associated with captivity occurs in some sea otters." In spite of these limitations, this is the largest and most detailed histopathologic evaluation of a marine mammal species exposed to an oil spill.

Petroleum hydrocarbons include a wide variety of materials, including crude oils, various refined products (kerosene, gasoline, mineral seal oil, naphtha), natural gas, and liquified petroleum gas.[12,28] These materials are complex mixtures of numerous hydrocarbons and various contaminants and additives. Crude oils

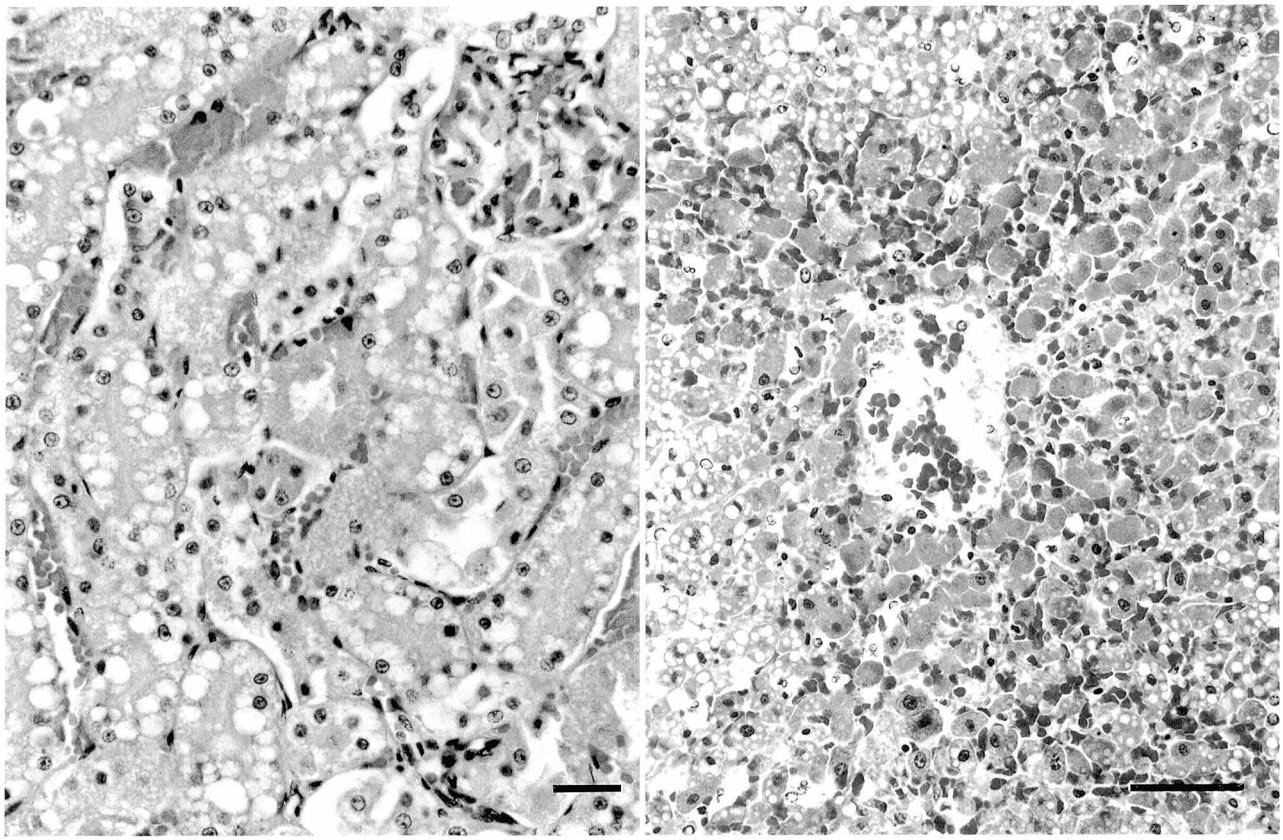

Fig. 4. Kidney; otter No. II , lipidosis of the proximal and distal tubular epithelium. HE. Bar = 25 μm.
Fig. S. Liver; otter No. 18, eentrilobular necrosis and midzonal lipidosis. Central vein is at center. HE. Bar = 50 μm.

collected from different sites contain various types and proportions of hydrocarbons. Thus, comparisons of toxicity of various petroleum hydrocarbon materials must be made cautiously." Nevertheless, as a practical matter, the toxicity of a particular petroleum material must be assessed in relation to what is known about related materials.

Aspiration pneumonia is the most common serious consequence of petroleum hydrocarbon ingestion in human beings and animals.v"-" In human beings, hydrocarbon toxicosis typically results from ingestion of petroleum distillates such as kerosene, gasoline, mineraI seal oil, lighter fluid, dry cleaning fluids, and petroleum solvents.':' Lesions in fatal human cases include necrosis of bronchial, bronchiolar, and alveolar tissues, atelectasis, interstitial inflammation, hemorrhagic pulmonary edema, vascular thromboses, necrotizing bronchopneumonia, and hyaline membrane formation." Complications include pneumatoceles,' pleural effusion, pneumothorax, pneumomediastinum, pneumopericardium, and subcutaneous emphysema.> Pneumatoceles are air-filled cavities within the pulmonary parenchyma. Their development after hydrocarbon ingestion is postulated to be caused either by overdistension and rupture of alveoli secondary to formation of one-way valve mechanisms in inflamed bronchi or by coalescence of abscesses or areas of necrosis that eventually penetrate a bronchial wall, thereby allowing the production of an air-containing cavity.' Oil-contaminated sea otters attempt to remove oil by grooming," which involves use of the mouth; this process would seem to provide ample opportunity for aspiration. However, no evidence of aspiration pneumonia, bronchitis, bronchiolitis, pulmonary abscesses, or areas of pulmonary necrosis were found in the sea otters.

Interstitial pulmonary emphysema was prevalent in oil-contaminated sea otters that died in rehabilitation centers (19/46). The lesion was more frequent in the more extensively contaminated otters. It was also present in 1/5 otters found dead at the site of the spill with external oil present. Emphysema was not seen in uncontaminated otters that died in rehabilitation centers nor in apparently normal otters. Dyspnea was common in oil-contaminated sea otters presented to rehabilitation centers; interstitial and subcutaneous emphysema was diagnosed by others in many of these sea otters." Although not reported in sea otters or other

animals contaminated by oil spills prior to the *Exxon Valdez* disaster, it is clear that exposure to crude oil causes sea otters to develop emphysema. The pathogenesis of the lesion in this setting is unclear. Alveolar tears are the usual route by which air enters the pulmonary interstitium.[10] Alveolar tears can occur when there is a combination of forced expiration or coughing and bronchiolar obstruction that produces sharply increased pressures within alveoli.[10] In anatomically predisposed species such as cattle, the lesion may occur agonally, presumably due to forced expiration combined with bronchiolar collapse. Predisposing factors include well-developed interlobular septa, lack of collateral ventilation, and greatly uneven deflation among adjacent lobules.[13] A cow that died after ingesting used motor oil had interstitial, alveolar, and subpleural emphysema,[7] yet emphysema was not described by researchers who gave lethal doses of crude oil orally to cattle.[3] Interstitial pulmonary emphysema has been reported in sea otters with pneumonia[4] and has been seen rarely as a mild focal lesion in sea otters that died without evidence of respiratory disease or oil exposure (T. P. Lipscomb, personal observation). Sea otters have well-developed interlobular septa and thus may be anatomically predisposed to development of interstitial emphysema, but exposure to crude oil resulted in a remarkably high incidence of the lesion. During the early days of the spill, inhalation of volatile components of crude oil such as benzene might have damaged alveolar septa and caused the lesion, but neither interstitial pneumonia nor other lesions that might result from inhalation of an irritant vapor were found in affected sea otters. Interstitial pulmonary emphysema has not been reported in experimental animals exposed to benzene or other volatile petroleum hydrocarbons by inhalation or other routes.[5,23,32,36,41] Thus, the pathogenesis of interstitial pulmonary emphysema in oil-contaminated sea otters is undetermined.

Gastric erosions were common (13/40) in oil-exposed sea otters that died in rehabilitation centers and were also found in one of the six uncontaminated otters that died in the centers. An explanation for the relatively low incidence in heavily contaminated Group No. 1 otters (Table I) is not readily apparent. The lesion was present in some sea otters that died within the first several days after arrival at the centers, but it was also seen in otters that died weeks or months after arrival. In cattle given crude oil orally, the only alimentary tract lesion found was black staining of the ruminal mucosa, which was presumed to represent a residue of the crude oil.[3] Similarly, birds given crude oil orally failed to develop alimentary tract lesions.[41] However, other studies report that crude oil and other petroleum hydrocarbons cause extensive damage to the alimentary tract including inflammation, erosions, hemorrhages, and marked vascular congestion.[5,23] Melena was reported in many otters in the rehabilitation centers.[4] Rapidly developing gastric erosions that appear following severe stress occur in human beings and animals.[17] Gastrointestinal erosion/ulceration and hemorrhage have been reported in sea otters that died in captivity and in the wild and have been attributed to stress.[4,17] All of the gastric erosions seen in this study were acute; none showed signs of healing. Those present in otters that died shortly after arrival at the rehabilitation centers might have developed prior to capture because of stress associated with oil exposure, as a direct effect of oil on the gastric mucosa, or because of stress associated with capture and captivity. Erosions caused by ingestion of corrosive liquids are extensive,[15] but the erosions encountered in these otters were small, discrete, and confined to the stomach. Thus, the erosions were probably caused by stress. Those seen in otters that died several days or more after arrival at the centers clearly developed in captivity.

Hepatic lipidosis was common in oil-contaminated otters that died in rehabilitation centers (14/47) and in oil-contaminated otters that were found dead (3/5). The lesion was more frequent in the more extensively contaminated otters, being found in 13/22 heavily and moderately contaminated sea otters that died within 8 days of arrival at the centers, and was not seen in otters that died later. It was also seen in an uncontaminated otter that died in a rehabilitation center (1/6). Renal lipidosis was somewhat less common and occurred only in otters that also had hepatic lipidosis. All animals with hepatorenal lipidosis were female except for one oil-contaminated male that was found dead. The sex ratio for Group No. I otters is I male : 2.6 females and for Group No. 2 otters is 1 male: 1 female. When the oil spill occurred, many females were in late gestation or had recently given birth. Hepatic and renal lipidosis have various causes, including toxins, mobilization of stored fats due to inadequate food intake, and hypoxia.[18-20] Researchers that applied crude oil to sea otters' coats reported marked increases in activity and metabolic rate with unchanged or decreased time devoted to feeding.[4] Animals with high energy demands such as those that occur during peak lactation or late gestation are predisposed to hepatic lipidosis.[18] Thus, hepatic and renal lipidosis may have been caused by an oil exposure-associated increase in energy demand with constant or decreased food intake, resulting in mobilization of stored fat. Some affected otters were further predisposed because of high energy demands due to pregnancy. Hepatic lipidosis in pregnant females may have been "physiologic," as occurs in ruminants, although this phenomenon has not been reported in sea otters. The fatty liver of hepatocellular hypoxia primarily affects centrilobular hepatocytes,[18] but lipid

accumulation in these otters was predominantly periportal. A direct or metabolite-associated toxic effect is another possible cause. Hepatic lipidosis has been reported in rats,' mice," cattle," sheep,ı and a ringed seal" exposed to petroleum hydrocarbons, but the mechanism was not determined. The pattern of distribution was variable, being periportal in the rats, diffuse in the mice, and centrilobular in the sheep; patterns were not reported in the cattle and the seal. Lipidosis of the proximal tubular epithelium of the kidney, also of undetermined pathogenesis, has been described in hydrocarbon-exposed rats.' Renal tubular epithelial necrosis, not further specified as to the affected segment of the nephron, has been reported in human beings," rats," and ringed seals" exposed to petroleum hydrocarbons but was not found in the sea otters. The high incidence of lipidosis in contaminated otters that died during the first few days of captivity, its presence in 3/5 contaminated otters that were found dead in the wild, and the absence of reports of lipidosis in otters that died in captivity suggest that captivity was not the cause of the lesion in these otters.

Centrilobular hepatic necrosis was also relatively common in oil-contaminated otters that died in rehabilitation centers (l 1/47) and was not found in uncontaminated otters that died in the centers. The lesion was present in some otters that died within the first few days after arrival at the centers, but it was also seen in otters that died weeks or months after arrival. Causes of centrilobular hepatic necrosis include toxins and conditions that cause hepatic ischemia, such as anemia, heart failure, and shock." Some oil-contaminated otters became anemic while at rehabilitation centers;" this anemia may have contributed to the development of centrilobular necrosis. Crude oil ingestion'? and gastric erosion with hemorrhage are possible causes of anemia; however, gastric erosions and centrilobular hepatic necrosis were found infrequently in the same otter, so anemia due to gastric hemorrhage was not a common cause of centrilobular hepatic necrosis. Other lesions likely to occur in heart failure were not found. Centrilobular hepatic necrosis of undetermined cause has been described by researchers who gave crude oil orally to birds." Many otters probably experienced shock." Multifocal hepatic necrosis and focally extensive hepatic necrosis that suggested infarction occurred at low frequency in both oil-contaminated and uncontaminated sea otters that died in rehabilitation centers. The causes of these lesions were not determined.

Sea otters are largely dependent on the insulating properties of their pelage for protection from the cold water they inhabit. It had been suspected that hypothermia would be a major problem in oil-contaminated sea otters because oil markedly increases the thermal conductance of their coats," and indeed hypothermia was a common problem in oil-contaminated sea otters presented to rehabilitation centers." Death caused by hypothermia can occur without distinctive gross or microscopic lesions." Stress and shock probably were significant medical problems." Both oil exposure and captivity are stressful to sea otters.P-" Hypothermia, stress, shock, respiratory compromise associated with interstitial emphysema, hemorrhage from gastric erosions, and hepatic necrosis all contributed to the deaths of oil-exposed sea otters.

In summary, interstitial pulmonary emphysema, gastric erosion and hemorrhage, hepatic and renal lipidosis, and centrilobular hepatic necrosis were common in oil-contaminated sea otters that died in rehabilitation centers and were absent or uncommon in the small group of uncontaminated sea otters that died in rehabilitation centers. These lesions were not seen in apparently normal, uncontaminated sea otters and, with the exception of gastric erosion and hemorrhage, have not been reported previously in association with death in captivity. Additionally, pulmonary interstitial emphysema and hepatic and renal lipidosis were present in a small group of oil-contaminated sea otters that were found dead in the wild. Pathologic examination of larger numbers of both oil-contaminated and uncontaminated sea otters not held in captivity would be useful in separating lesions resulting from exposure to crude oil from those resulting from effects of captivity.

## Acknowledgements

We thank D. Bruden, R.-A. V. Ferris, and M. A. Bers for technical assistance. The opinions and assertions contained herein arc those of the authors and are not to be construed as official or representing those of the Department of the Army, the Department of Defense, the Environmental Protection Agency, or the US Fish and Wildlife Service. MAJ T. P. Lipscomb, LTC R. B. Moeller, and COL J. M. Pletcher are members of the US Army. LtCol R. K. Harris is a member of the US Air Force.

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