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CHRONIC EFFECTS OF THE *EXXON VALDEZ* OIL SPILL ON BLOOD AND ENZYME CHEMISTRY OF RIVER OTTERS

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Abstract—River otters (*Lutra canadensis*) living in marine environments of Prince William Sound, Alaska, and exposed to crude oil from the *Exxon Valdez* spill in March 1989 showed elevated levels of blood haptoglobins, and interleukin-6 ir, as well as elevated activities of aspartate aminotransferase, alanine aminotransferase, and creatine kinase in summer 1991. Stepwise logistic regression, using a subset of these and other blood proteins and enzyme activities as potential independent variables, correctly classified 86.4% of 22 otters as inhabiting oiled or nonoiled areas. River otters abandoned latrine sites (an index to their abundance) over three times more often in oiled than in nonoiled areas, suggesting there may have been a delayed response in river otter populations to exposure to crude oil. Ours is the first clear model for the long-term effects of an oil spill on blood parameters of a free-ranging mammal using a nonlethal methodology. These effects occurred two years after the spill and following a major effort to clean oil from the shorelines of Prince William Sound.

Keywords—*Lutra canadensis* *Exxon Valdez* oil spill Haptoglobins Blood enzymes Chronic effects

INTRODUCTION

River otters (*Lutra canadensis*) are widely distributed along coastal shores of the Pacific Northwest, including Prince William Sound, Alaska. As near-shore foragers on marine fishes and invertebrates in intertidal and subtidal zones [1,2], they are potentially excellent indicators of marine pollution such as that from the *Exxon Valdez* oil spill of March 1989 [3,4]. In that spill, over 11 million gallons of North Slope crude oil was carried over more than 3,500 km of shoreline in Prince William Sound (Fig. 1). We previously documented [5] that one year after the oil spill and an extensive effort to clean shorelines, river otters from a heavily oiled area (Herring Bay on northern Knight Island) had lower body mass and higher levels of blood haptoglobins than otters inhabiting a nonoiled area (Esther Island; Fig. 1). We hypothesized that elevated haptoglobin levels over so long a period could indicate chronic inflammation and liver injury, or be related to the hemolytic anemia observed in birds [6,7] and sea otters (*Enhydra lutris*) [8] after acute exposure to oil. Nevertheless, because otters initially were live-trapped over about 80 km of shoreline in both Herring Bay and Esther Passage, we could not rule out some localized effect having caused the elevated blood parameters for otters in the sample from the oiled area. Consequently, we extended our sampling over large areas of Prince William Sound to test the hypothesis that river otters inhabiting oiled areas could be discriminated from those living in nonoiled areas, based on a suite of blood measurements. In summer 1991, two years after the oil spill, we live-trapped river otters from throughout Prince William Sound to test for elevated levels of blood haptoglo-

bins, and to examine additional blood parameters related to hemolytic anemia and immune-system function.

METHODS

We sampled shorelines extensively for the presence of otter latrine sites at Squire Island, Snug Harbor on southern Knight Island, Sleepy Bay on LaTouche Island, and Shelter Bay on Evans Island, Prince William Sound. Latrine sites provide an index to the density of otters [9]. Evidence of recent use (fresh scats, disturbed vegetation) was used to classify sites as active or abandoned. We live-trapped river otters from sites that showed heavy use by these mammals. Haptoglobin (Hp) in blood samples of river otters was quantified by electrophoresis as Hp-hemoglobin complexes. Hemoglobin (Hb) was added to the serum (1 to 20 ratio of hemoglobin [10% solution] to serum sample). The sample mixture was then electrophoresed on agarose gels at 100 V for 1 h, then the protein complex was fixed with 7.5% trichloroacetic acid. Gels were stained for Hb using *o*-dianisidine (Helena Laboratories, Beaumont, TX; tech. bull. no. 5445). The Hp-Hb complex, which migrates in a different region from Hb, is quantitated by densitometry, and results are expressed as milligrams of hemoglobin-binding capacity per 100 ml serum [10]. The accuracy of Hp quantification is based on the specificity of the Hp-Hb complex formation, which is separated by electrophoresis. The reproducibility of the Hp assay from comparison samples in previous Hp assays of river otters was indicated by interassay C.V. of 14.8%. This C.V. is similar to those reported in analyses of dog and horse Hp [11,12]. Interleukins (IL-6 ir, IL-1 ir) were determined by ELISA assay (R. and D. Systems, Minneapolis, MN). The interassay percentage C.V. for the interleukin assay as described by R and D Systems was 7.1% at the 90-pg/ml level. Samples run

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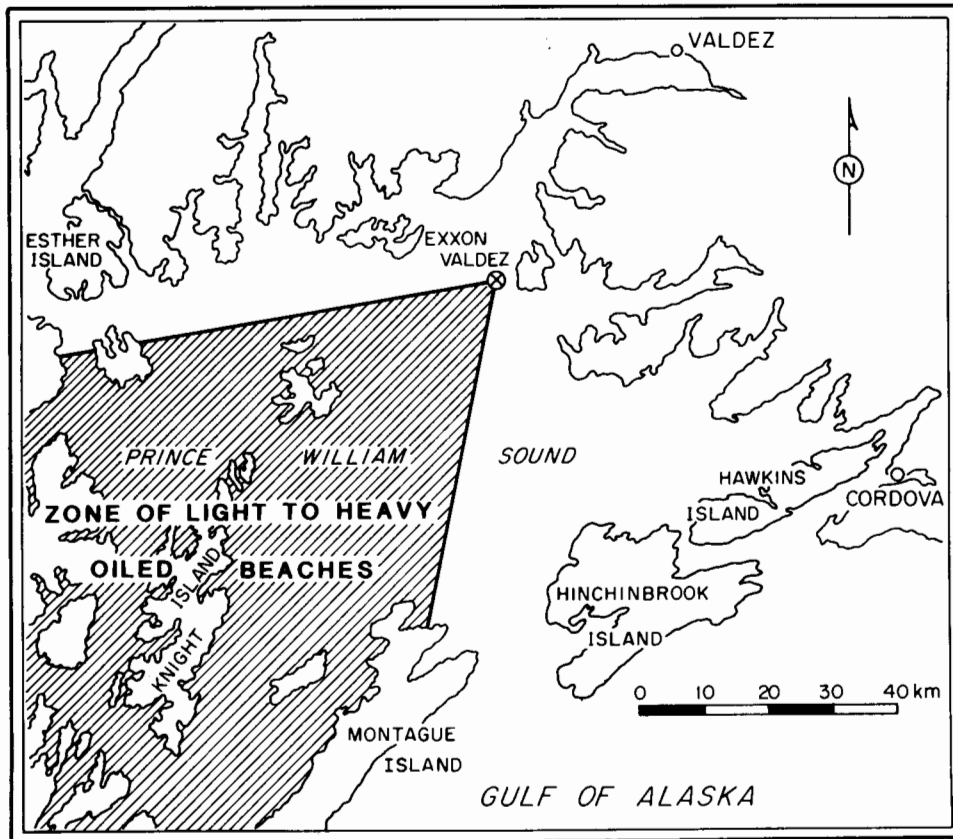


Fig. 1. Areas of Prince William Sound, Alaska, affected by the *Exxon Valdez* oil spill. River otters were live-trapped from nonoiled areas at Olsen Bay (northwest of Cordova) and Unakwik Inlet (west of Esther Island). Otters were live-captured from the heavily oiled areas of northern Knight Island, Eleanor Island, and Naked Island.

in duplicate were added to a microtiter plate coated with a monoclonal antibody for IL-6. After washing away any unbound proteins, an enzyme-linked polyclonal antibody for IL-6 was added to the wells and incubated to allow for binding any IL-6 in the wells. After a final wash, a substrate solution was added to the wells, and color developed. Concentrations were determined from a standard curve. Enzyme concentrations were obtained using standard clinical auto-analyzer procedures at Fairbanks Memorial Hospital, Alaska. In all cases, a single analysis was performed, except for the interleukins described above.

We examined relationships among blood values with simple correlations [13]. These and other blood parameters were used to classify otters from oiled and nonoiled areas using logistic regression [13,14]. The binomial response (oiled or unoiled) takes the form

$$\pi(x) = \frac{\exp(\alpha + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_K X_K)}{1 + \exp(\alpha + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_K X_K)}$$

where π is the probability that an otter came from an oiled area, and α and β are regression coefficients of the logit transformation [14]

$$\log\left(\frac{\pi(x)}{1 - \pi(x)}\right) = \alpha + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_K X_K.$$

RESULTS

A difference in Hp levels was observed between otters living in oiled and nonoiled environments (Table 1). Also, increases were noted in creatine kinase (CK), aspartate aminotransferase (ASAT), and alanine aminotransferase (ALAT) in river otters living in oiled areas of Prince William Sound. Besides liver function, an effect on the immune system was noted by increased levels of IL-6 ir, and a slight increase in mean levels of IL-1 ir.

Stepwise logistic regression, with 26 blood values as potential independent variables, selected only three blood variables – Hp, ASAT, and IL-6 ir – to correctly classify 86.4% of 22 river otters as coming from oiled (coded 0) or nonoiled (coded 1) areas of Prince William Sound. The resulting model,

$$\log\left(\frac{\pi(x)}{1 - \pi(x)}\right) = 5.1280 - 0.2886 \text{ Hp} - 0.1043 \text{ ASAT} \\ - 0.1724 \text{ IL-6 ir},$$

Table 1. Selected blood parameters for river otters inhabiting oiled ($n = 11$) and nonoiled ($n = 11$) areas of Prince William Sound, Alaska, in 1991

Blood parameters	Oiled		Nonoiled	
	\bar{X}	SE	\bar{X}	SE
Interleukin (IL-6 ir, pg/ml)	48.3	13.8	17.3	11.3
Interleukin (IL-1 ir, pg/ml)	13.3	6.6	10.1	6.1
Haptoglobin (Hp, Hb binding/dl)	156.9	27.9	30.0	15.6
Alanine aminotransferase (ALAT, IU/L)	152.7	8.8	138.5	14.6
Aspartate aminotransferase (ASAT, IU/L)	437.2	70.0	418.1	67.0
Lactate dehydrogenase (LDH, IU/L)	146.2	25.2	154.0	43.1
Creatine kinase (CK, IU/L)	3,038.6	820.8	1,885.8	516.4
Hemoglobin (Hb, g/dl)	16.3	0.6	15.7	0.6
Packed cell volume (PCV, ml/mm ³)	42.9	1.6	44.1	1.6

did not depart from a logistic fit, as determined by a Hosmer-Lemshow goodness-of-fit chi-square ($P = 0.27$). Sex, body length, or mass of otters, along with the other blood parameters, failed to improve the fit of this model. ASAT also brings information to the model about other enzymes; ASAT was positively correlated with ALAT ($r = 0.52$) and CK ($r = 0.86$). Thus elevated levels of Hp, ASAT, and IL-6 ir from otters in oiled zones likely indicate chronic inflammation and liver damage continuing two years following the oil spill.

River otters abandoned significantly more latrine sites (i.e., showed no evidence of recent use) in oiled than in nonoiled areas of Prince William Sound (Fig. 2). This threefold difference between oiled and nonoiled areas suggests that other populations are continuing to decline in oil-exposed regions.

DISCUSSION

An acute-phase response, anemia, and increased creatine kinase were reported for sea otters exposed to oil in Prince William Sound in 1989 [8]. In 1990, we observed elevated levels of the acute-phase protein, Hp, in river otters from oiled areas [5]. In 1991, we continued to observe a difference in Hp levels between river otters living in oiled and nonoiled environments. We did not observe the hemolytic anemia reported for birds ingesting large amounts of crude oil [6,7] and believe acute anemia is an unlikely explanation for elevated levels of Hp in river otters because neither Hb nor packed cell volume were significantly lower in otters from oiled areas (Table 1). Moreover, a preliminary examination of blood slides from river otters from summer 1991 revealed no obvious abnormalities, including Heinz bodies (A. Rebar, personal communication). All these blood characteristics, symptomatic of Heinz-body hemolytic anemia in birds [7], were absent in otters. Additionally, as reported by Wilson et al. [8] in two recovering sea otters, hemocrits returned to the normal range after three months following exposure to crude oil. Many of those recovering sea otters, however, did not survive long after release [15].

Variation in Hp levels is one of a large number of systemic and metabolic changes related to inflammation that is coordinated by cytokines. Many different cytokines have over-

lapping activities and can bring about similar effects in the same or different cells, as well as acting synergistically with other cytokines and hormones [16]. Hp synthesis is stimulated by IL-6, and this stimulation can be modulated by other cytokines [16]. ASAT, ALAT, and CK normally occur on the inside of cells but due to leakage appear in low amounts in the blood. This leakage is increased during cell damage and necrosis; measured enzyme activity provides a sensitive indicator of disease or stress [17,18]. ASAT and ALAT are traditionally used to monitor liver disease, whereas increased CK

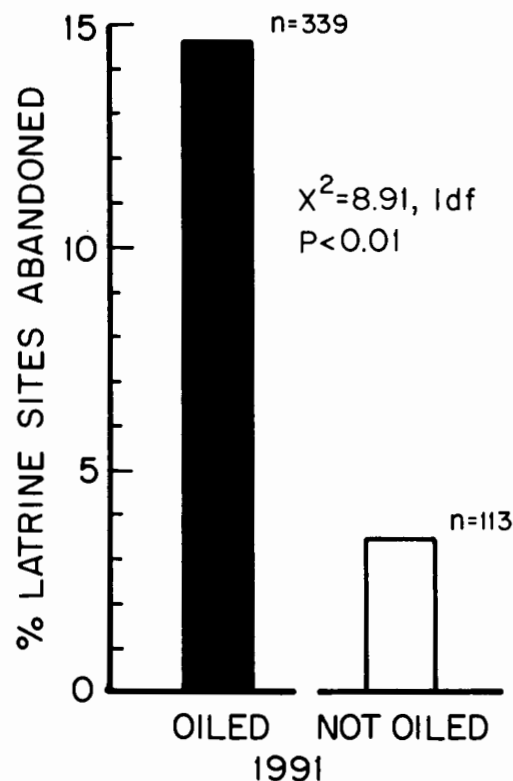


Fig. 2. Percentage of river otter latrine sites abandoned on oiled and nonoiled areas of Prince William Sound, Alaska, summer 1991.

indicates damage to skeletal muscle and the central nervous system. Oil ingested in food or while grooming [8,15] would lead to oil in the blood and tissue inflammation. Increases in CK, ASAT, and ALAT as well as Hp and IL-6 ir levels could indicate that the liver and immune system are being stressed [17–19]. Elevations in ALAT have recently been reported in mink fed PCBs [16], and these changes have indicated a lessening of the integrity of hepatocytes. Cytokines such as interleukins also have been implicated in demyelinating brain lesions [20].

Long-term systemic effects of oil require absorption through the gastrointestinal tract [4] and the mucosal cells that line the tract. Some chemicals present in crude oil may become involved in enterohepatic recycling, which is capable of prolonging their stay in the animal. River otters would introduce oil toxicants into the gastrointestinal tract by eating prey [9] and grooming pelage [4,15]. In most cases, the primary site of bioaccumulation of complex organic mixtures in mammals is the lipid fractions, as reviewed by Talmage and Walton [21].

Hp values for river otters obtained in 1991 are substantially lower than those from 1990 [5]. This decrease may have occurred for three reasons: First, the Hp response elicited by exposure to crude oil may be waning over time; second, samples were collected in late winter in 1990 but during summer in 1991; and third, we previously excluded very low Hp values from areas without oil from our analysis because we suspected these samples might have been outliers [5]. Although the effects of exposure to oil would likely diminish over time, it would not explain why Hp levels declined markedly from 1990 levels on both oiled and nonoiled areas. Likewise, omitting low Hp values, which we know to be valid, explains neither the magnitude of this change nor its occurrence on oiled areas (where we deleted no samples). Thus, the most likely answer is that Hp levels are the result of complex interactions with other environmental factors that vary seasonally. River otters gain body mass as seasons change from stressful winter conditions to a more hospitable climate with abundant foods in summer [5]. Moreover, river otters mate during late winter, when they were sampled in 1990.

Elevated levels of IL-6 ir in otters exposed to oil and its prominence in our statistical model suggest that such otters may be suffering from impaired immune systems [19,20,22,23], which would predispose otters to disease long after the oil spill and attempts to clean oil from contaminated shorelines. Moreover, we suggest that carefully controlled lab experiments might not reveal this process because of difficulties in recreating the combination of natural conditions such as variations in temperature, daylight, food, and so forth, or in exposing otters to potential infectious agents normally occurring in the environment [19]. We hypothesize that exposure to oil might lead to recurrent bouts of infection and inflammation leading to reduced survivorship or reproduction of individual otters. Our data on the significantly higher rate of latrine-site abandonment (Fig. 2) in oiled areas support this interpretation.

Clearly, there is a need for more baseline data on otters (and other vertebrates) so that pre- and postoil-spill periods can be compared. Such data were unavailable at the start of our research. Likewise, there is a critical need to continue

monitoring blood values from otters in Prince William Sound. Finally, we encourage others studying the effects of oil exposure on vertebrates to examine chronic as well as acute effects, and to consider blood parameters such as acute-phase proteins like Hp and cytokines like IL-6 as possible indicators of oil exposure, especially for the recent oil spills in Spain and Scotland. We caution investigators, however, to consider how these blood values may interact with environmental factors and that there may be differences among species. We believe our modeling approach offers an important nonlethal method to evaluate oil exposure in a natural setting.

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