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26 May 1977

Ingestion of Crude Oil: Sublethal Effects in Herring Gull Chicks

Abstract. *A single small oral dose of Kuwait or South Louisiana crude oil caused cessation of growth, osmoregulatory impairment, and hypertrophy of hepatic, adrenal, and nasal gland tissue in herring gull chicks living in a simulated marine environment. These findings suggest that ingesting crude oil causes multiple sublethal effects that might impair a bird's ability to survive at sea.*

The total annual influx of oil to the oceans of the world was estimated to be between 5 and 10 million metric tons in the early 1970's and was considered to be increasing as a result of increased transportation and expanded exploitation of regions in which drilling might be more hazardous (*1*). Although spectacular oil spills account for only a small fraction of the total, the number of oiled sea and shore birds that wash up after a spill indicates that oil is acutely toxic. Furthermore, the low survival rate of apparently healthy birds that have been cleaned of oil and released suggests delayed toxic effects (*2*). These delayed effects could be caused by oil that is swallowed or aspirated when birds preen or ingest contaminated food and seawater (SW). Crocker *et al.* (*3*) have reported that the ingestion of crude oil inhibits salt and water transport in the intestine of saline-adapted ducklings. Although they did not measure plasma electrolytes, we have found that crude oil does impair plasma osmoregulation in SW-stressed (nonadapted) ducklings (*4*). These data suggest one possible mechanism of toxicity in a species that is not strictly marine. Laboratory experiments with true sea birds present many problems. Although most species of gulls are coastal, they can osmoregulate in a simulated marine environment (Fig. 1); they thus provide a convenient laboratory model for studying the toxicity of oil to sea birds. We now report that a single small dose of crude oil inhibits growth and impairs plasma osmoregulation in immature herring gulls (*Larus argentatus*). Crude oil also causes hypertrophy of adrenal, hepatic, and nasal gland tissue and induction of hepatic microsomal cytochrome P-450 activity.

Herring gull chicks about 3 to 4 weeks of age were captured on Old Man Island, off Cutler, Maine, and transported to the Mount Desert Island Biological Laboratory. Birds were housed in sheds and fed whole unsalted herring and 50 percent SW, which were freely available. After 5 days, birds were divided into two experimental groups and one control group of six birds each. Birds in the experimental groups were given by intubation a single oral, 0.2-ml dose (dose equivalent to 0.3 ml per kilogram of body weight) of either a Kuwait crude (KC) oil containing 22 percent aromatics or a heavier South Louisiana crude (SLC) oil containing 17 percent aromatics (*5*). This dose appears to be environmentally realistic and is well below that which causes lipid pneumonia in waterfowl (*6*). Experimental and control birds were then moved to separate sheds, and all were given 100 percent SW (440 meq per liter of Na^+, 9.4 meq per liter of K^+, and 940 mosmoles per kilogram of water to drink. Each day, gulls were weighed and 0.2-ml blood samples (wing vein) were taken. One day after being dosed, a control and an SLC gull died; 4 days later another SLC gull died after exhibiting

Table 1. Effect of crude oil (a single oral 0.2-ml dose) on herring gull intestinal transport, organ weights, and activity of Na^+,K^+-adenosine triphosphatase (Na^+,K^+-ATPase). Each value is given as the mean ± standard error of the mean; statistical comparisons are made between experimental and control groups and tested according to unpaired *t*-tests.

Item	Group		
	Control (N = 5)	KC (N = 6)	SLC (N = 4)
Intestinal transport†			
0.1 m*M* cycloleucine			
3 minutes	163 ± 19	178 ± 14	115 ± 9*
10 minutes	337 ± 34	395 ± 66	243 ± 17*
0.1 m*M* cycloleucine + 10 m*M* leucine			
3 minutes	89 ± 4	83 ± 8	83 ± 3
Inhibition (%)	43 ± 5	48 ± 7	27 ± 4*
1 m*M* glucose			
3 minutes	1865 ± 232	2181 ± 320	1199 ± 315
1 m*M* glucose + 0.1 m*M* phlorizin			
3 minutes	883 ± 114	915 ± 56	771 ± 28
Inhibition (%)	49 ± 9	53 ± 8	29 ± 14
Intestine			
Weight/surface area‡	4.48 ± 0.14	4.23 ± 0.17	4.14 ± 0.33
Na^+,K^+-ATPase activity (specific)			
Weight§	2.82 ± 0.40	2.32 ± 0.62	1.76 ± 0.61
Area‖	2.50 ± 0.30	2.22 ± 0.56	1.64 ± 0.94
Nasal salt gland (one)			
Gland weight			
Weight (mg)	387 ± 22	436 ± 28	451 ± 32
Weight/body weight (mg/kg)	454 ± 17	563 ± 14**	575 ± 37*
Na^+,K^+-ATPase activity			
Weight (specific)§	47 ± 2	36 ± 1**	32 ± 2**
Gland (total)¶	1037 ± 74	908 ± 76	817 ± 50*
Liver			
Organ weight			
Weight (g)	22.3 ± 1.4	29.6 ± 2.8*	32.6 ± 2.0**
Weight/body weight (g/kg)	0.26 ± 0.01	0.38 ± 0.02**	0.42 ± 0.01**
Cytochrome P-450 activity#	420 ± 80	780 ± 120*	910 ± 130*
Adrenal glands (two)			
Gland weight			
Weight (mg)	83 ± 4	95 ± 13	103 ± 6*
Weight/body weight (mg/kg)	0.98 ± 0.08	1.27 ± 0.17	1.33 ± 0.11*

†Nanomoles per square millimeter of tissue. ‡Milligrams per square millimeter. §Micromoles of inorganic phosphate (P_i) per milligram of protein per hour. ‖Micromoles of P_i per square millimeter per hour. ¶Micromoles of P_i per gland. #Nanomoles per milligram of protein. *$P < .05$. **$P < .01$.

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extremely high concentrations of Na^+ in the plasma. Because of the small sample size, we could not determine whether crude oil was responsible for the observed mortalities. After 8 to 9 days, birds were decapitated; liver, nasal glands, adrenals, and tissue from the small intestine were excised and weighed; samples of intestinal tissue were fixed for histology (*7*). The uptakes of [^{14}C]cycloleucine and [^{14}C]glucose were determined in intestinal slices by the procedure of Miller *et al.* (*8*). The activity of Na^+,K^+-adenosine triphosphatase (Na^+,K^+-ATPase) was assayed in freeze-dried homogenates of nasal gland and intestinal mucosa as previously described (*9*, *10*). Cytochrome P-450 activity was determined in microsomal fractions of liver samples by the procedure of Omura and Sato (*11*). Protein was determined by the procedure of Lowry *et al.* (*12*), in which crystalline serum albumin is used as the standard. Plasma Na^+ and K^+ concentrations were determined by flame photometry.

During the period of SW maintenance, control gulls gained weight at a rate comparable to that reported for herring gull chicks in the wild (*13*), about 3 percent per day (Fig. 1); in contrast, gulls given oil gained no weight during this period. To determine whether the failure of the experimental gulls to gain weight was a result of loss of appetite, we estimated food intake as the difference between the weight of fish presented and that remaining after each feeding. Since KC and SLC gulls were housed together, we could calculate only mean food intake for all treated birds. Over 3 days, control birds ingested an average of 360 g of fish per bird, and experimental birds ingested 414 g. Since experimental gulls consumed 15 percent more fish than control gulls, their failure to gain weight cannot be attributed to loss of appetite. To determine whether intestinal nutrient absorption was impaired in oil-treated birds, we incubated intestinal slices in oxygenated physiological saline containing [^{14}C]cycloleucine (a nonmetabolizable analog of the essential amino acid leucine) or D-[^{14}C]glucose. Previous studies with avian (chicken) intestine (*14*) have shown amino acid and hexose transport exhibit saturation kinetics and are concentrative (steady-state tissue-to-medium ratios exceed unity), Na-dependent, and inhibitable by structural analogs or by the glycoside phlorizin (hexose only). In control gulls, the transport of cycloleucine and glucose was substantially inhibited by leucine or phlorizin, respectively (Table 1). Intestinal tissue from SLC gulls accumu-

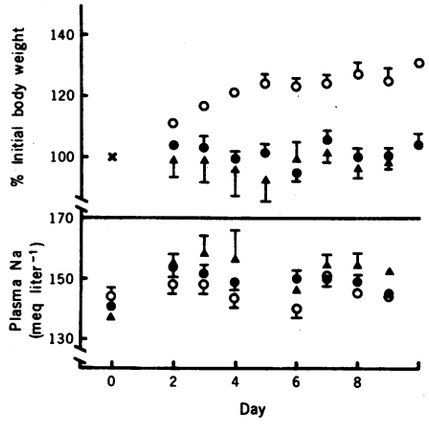
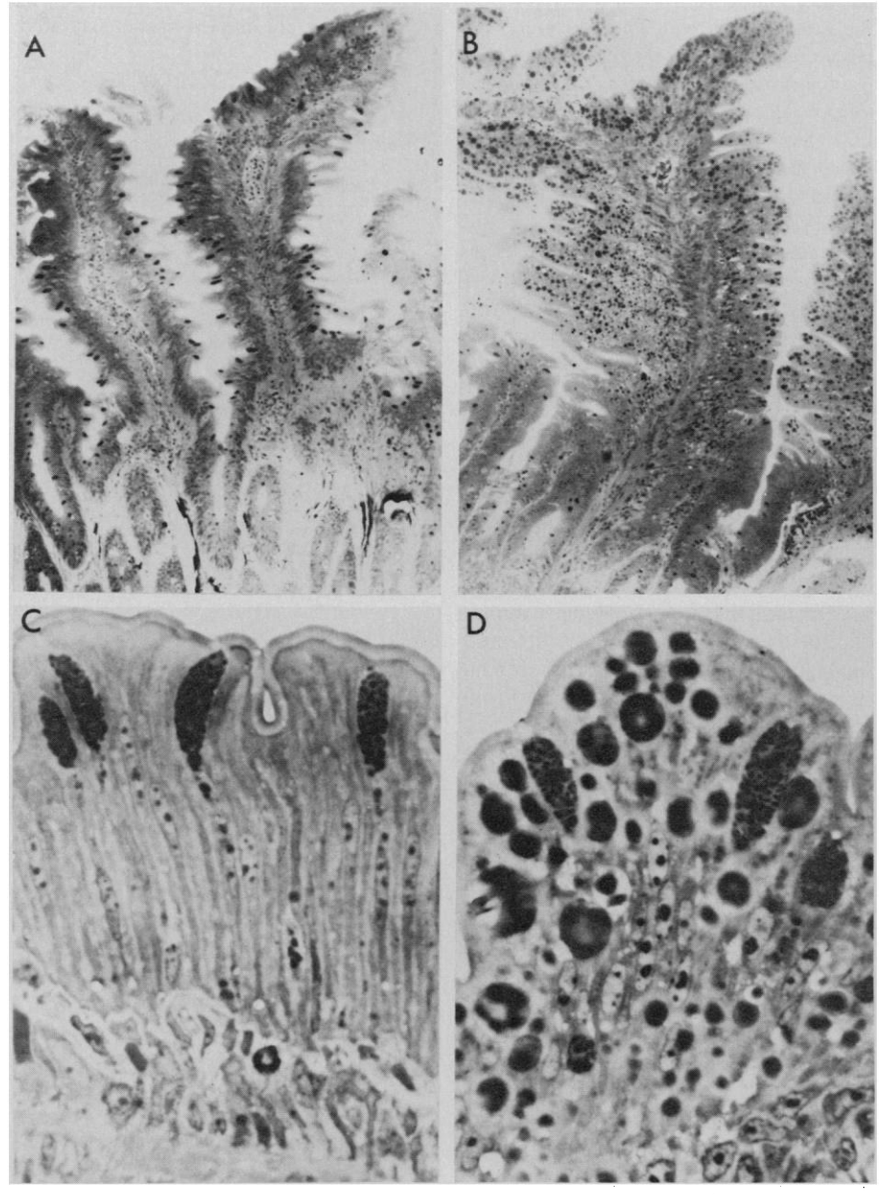


Fig. 1 (left). Effect of a single oral 0.2-ml dose of Kuwait (•) or South Louisiana (▲) crude oil on body weight and plasma Na^+ concentrations in herring gulls maintained on seawater for 0 to 8 days after being dosed. Each point represents the mean value derived from four KC, five control (○), or six SLC birds; when large enough, standard errors are represented by bars. Body weight for treated gulls was significantly ($P < .05$, unpaired t-test) lower than control gulls after day 2. Plasma Na^+ was significantly higher for the KC than for the control group on day 6; SLC plasma Na^+ was significantly higher than that for the control group on days 6, 8, and 9. Fig. 2 (right). Light micrographs of tissue from the small intestines of control (A and C) and SLC-treated (B and D) gulls. Intestinal slices were fixed in glutaraldehyde, exposed to osmium vapor, and embedded in Epon. Plastic sections were stained with methylene blue–azure II and photographed with phase contrast optics. Magnification: A and B, ×115; C and D, ×950.



lated about 30 percent less cycloleucine than that from control gulls. Only the mediated (that is, leucine inhibitable) component of uptake was affected. Glucose accumulation by SLC gull intestine was 35 percent less than that of control gulls; however, this decrease was not statistically significant because of the large sample variability and small sample size. Nutrient uptake by KC gull intestine was not different from control values (Table 1). Light micrographs of the small intestine of SLC gulls revealed major pathological changes in tissue morphology, which might be related to the impaired nutrient transport observed. Intestine from gulls treated with SLC exhibited proliferative edema with considerable cytoplasmic disruption (Fig. 2B). Numerous dark-staining bodies were present in the epithelial mucosa. These bodies were Sudan Black–positive, suggesting droplets of lipid (*15*). Light micrographs made at higher magnification (Fig. 2) showed these droplets to be located within the cytoplasm of the epithelial cells. Droplets were also found in tissue sections from one of the two KC gull intestines examined, but droplet density was much lower than in SLC tissues. Beer (*16*) has reported that intestinal tissue from severely oiled sea birds exhibits acute enteritis. Although we observed no evidence of enteritis in our gulls, the morphological changes we report could represent an early stage or a mild form.

During the period of SW maintenance, plasma Na^+ concentrations in control birds remained constant (Fig. 1). In contrast, KC Na^+ concentrations tended to be slightly elevated, and SLC concentrations were significantly higher than those of controls on days 6, 8, and 9 (Fig. 1); plasma K^+ was not affected by crude oil. These data indicate that salt and water balance was disrupted in experimental gulls and that osmoregulatory organs are possible targets of crude-oil action. Marine birds drinking SW osmoregulate by absorbing NaCl and water from the intestine and excreting excess salt through the nasal glands (*17*). Since the enzyme Na^+,K^+-ATPase is believed to take part in the active ion pumping process in both osmoregulatory organs (*18*), we assayed Na^+,K^+-ATPase activity in homogenates of intestinal mucosa and nasal gland. Mean intestinal Na^+,K^+-ATPase activities were reduced 17 and 38 percent in KC and SLC gulls, respectively (Table 1); however, because of the low enzyme activity in this tissue and the small sample size, experimental enzyme activities were not significantly different from those of controls. In the nasal gland, crude oil lowered the specific activity of Na^+,K^+-ATPase by 30 percent (Table 1). This reduction was accompanied by hypertrophy of nasal gland tissue so that total activity in the gland was not significantly reduced in KC gulls and was reduced by only 20 percent in SLC gulls. Similar compensatory effects were observed in the nasal glands of common puffins (*Fractercula arctica*) which had been treated with 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE) (*9*). The data on Na^+,K^+-ATPase (Table 1) parallel the effects of crude oil on plasma osmoregulation (Fig. 1).

Treatment with crude oil also caused significant hypertrophy of hepatic tissue and induction of microsomal cytochrome P-450 activity (Table 1). Although induction of the hepatic mixed-function oxidase system by environmental pollutants is well documented in many species (*19*), to our knowledge, this is the first report of induction by petroleum hydrocarbons in a sea bird. Finally, SLC, but not KC, caused adrenal gland hypertrophy in our gulls. Adrenal hypertrophy is generally recognized to be a reaction to nonspecific stress. However, with SLC gulls it may also reflect increased production of adrenocortical hormones with rising plasma Na^+ levels since these hormones are believed to stimulate adaptive hypertrophy of nasal gland tissue in response to an osmotic load (*17, 20*).

The data indicate that ingestion of crude oil impairs growth and osmoregulation in herring gull chicks. Inhibition of growth by SLC appears to be related, at least in part, to reduced nutrient transport in the intestine. There is considerable evidence that environmental pollutants such as organochlorines and heavy metals can also alter nutrient balance by disrupting metabolism and excretory transport (*21*). These mechanisms of toxicity may be of primary importance for KC-treated birds. During the summer of 1976, we performed limited experiments with a true oceanic species, the black guillemot (*Cepphus grylle*). We gave immature birds on Old Man Island a single oral 0.2-ml dose of KC (dose equivalent to 0.6 ml per kilogram of body weight) and drew blood samples 3 days later. Although sample size was too small to permit detailed analysis, experimental birds exhibited significantly smaller gains in body weight and slightly elevated plasma Na^+ concentrations when compared with controls from the same nest. Because of the small scale of our initial gull and guillemot experiments, we could not define the time course and dose response for each of the crude-oil effects observed. Nor could we begin to identify which of the many compounds in these extremely complex mixtures are responsible for these effects. Nevertheless, our data do indicate that small amounts of ingested crude oil produce multiple sublethal effects that could reduce a sea bird's capacity for long-term survival. When considered in conjunction with other stresses normally encountered by birds at sea (for example, food shortages and severe storms), the potential for lethality is considerable.

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22. Supported by PHS grant ES00920 and NSF grant GB 7851. We thank C. Bunker, E. Burnham, H. Church, and B. Peakall for excellent technical assistance. Analyzed reference oil samples were provided by the American Petroleum Institute.
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31 March 1977; revised 2 May 1977