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Heinz-Body Hemolytic Anemia from the Ingestion of Crude Oil: A Primary Toxic Effect in Marine Birds

Abstract. *Hemolytic anemia developed in young herring gulls and Atlantic puffins given daily oral doses of a Prudhoe Bay crude oil. Anemia developed 4 to 5 days after the initiation of oil ingestion and was accompanied by Heinz-body formation and a strong regenerative response. The data evince a toxic effect on circulating red blood cells involving an oxidative biochemical mechanism and the first clear evidence of a primary mechanism of toxicity from the ingestion of crude oil by birds.*

Petroleum oils regularly enter the marine environment through spills, runoff, and seepage (*1*). Large numbers of birds have died in association with marine oil spills (*2*), and the effects of oil on birds have been studied experimentally. Birds that become oiled ingest oil while preening (*3*), and oral doses of several petroleum oils have produced a wide range of sublethal toxic changes affecting growth, reproduction, osmoregulation, steroid metabolism, and hepatic function (*4–6*). Thus there is a firm basis for concern that oil pollution may produce subtle, sublethal effects in wild birds that impair reproduction or survival. This concern is heightened by the current emphasis on offshore oil development.

We report here that young herring gulls (*Larus argentatus*) and Atlantic puffins (*Fratercula arctica*) developed a severe hemolytic anemia after several days of oral dosing with a Prudhoe Bay crude oil (PBCO). Our data indicate that this was a primary toxic effect in which oxidative chemical processes damaged red blood cells in the peripheral circulation, and they constitute the first clear evidence of a primary toxic mechanism in experimental studies of the toxicity of ingested crude oil in birds.

In our initial experiments we used herring gull nestlings 2 to 3 weeks old, taken from a coastal colony. These young birds adjust well to captivity and tolerate the manipulation required in laboratory work. Gulls were collected on Great Island, 50 km south of St. John's, Newfoundland, and held in pens at Memorial University of Newfoundland, St. John's (*7*). Pens were partially bedded with hay, and the birds were fed unlimited amounts of capelin (*Mallotus villosus*) and seawater. When all the birds were gaining weight (3 to 5 days after capture), they were divided into experimental groups of similar mean body weight (460 g) and weight distribution. Blood (1 ml) was taken from each bird, and experiments were begun immediately. In experiment 1, the birds were given either 10 or 20 ml of PBCO (*8*) per kilogram of body weight per day in gelatin capsules; controls received empty capsules. Dosing continued daily. Blood samples were drawn into heparinized tubes on day 5, and routine hematological measurements were made (*9*). Values for all groups prior to dosing and for controls on day 5 of dosing did not differ significantly. On day 5, blood taken from oil-dosed birds was dark brown and failed to redden on mixing with air; this result suggested a marked reduction in oxygen-carrying hemoglobin. The birds receiving oil were severely anemic, with packed cell volumes (PCV) reduced by 43 and 50 percent (Table 1). The plasma from these birds was rusty red, an indication of hemolysis either intravascularly or during sample handling. A strong regenerative response to the anemia was evident in the high reticulocyte count (Table 1). After the birds were killed, there was no evidence of trauma, enteric bleeding, or other hemorrhage. These data are sufficient to permit the classification of this anemia arising from oil ingestion as a hemolytic anemia (*10*).

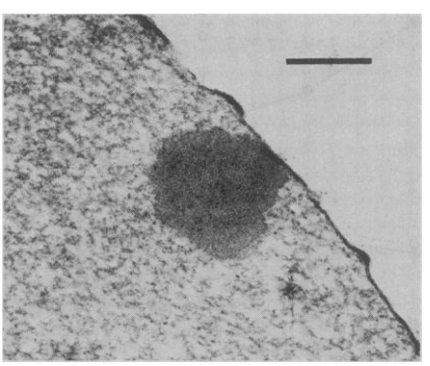

Fig. 1. Transmission electron micrograph of a Heinz body attached to the plasma membrane of a red blood cell from a herring gull which ingested 20 ml of Prudhoe Bay crude oil per kilogram per day for 4 days (experiment 1). This cell is a ghost erythrocyte, with most of the free hemoglobin lost from its cytoplasm; bar, 200 × 10^{-6} mm.

Heinz bodies were abundant in erythrocytes from oil-dosed birds (Table 1). These were identified by two different staining techniques applied to fresh samples and in sections studied by light and transmission electron microscopy (Fig. 1) (*9*). Heinz bodies are dense granular masses in red cells thought to consist of precipitates of hemoglobin oxidized in the protein moiety. They are a classical feature of toxic hemolytic anemias produced by a variety of dissimilar chemicals linked mechanistically by the ability to cause destructive oxidative reactions in red cells (*11*). The presence of Heinz bodies is good evidence of a primary toxicosis of red cells and of an oxidative biochemical mechanism of toxicity. To probe other possible sites of destructive oxidative processes in these red cells, we measured methemoglobin, sulfhemoglobin, and reduced glutathione (GSH) in whole blood and extractable fluorescence (EF) in red cell membranes as an indicator of membrane lipid peroxidation (*12*). No sulfhemoglobin was detected. The range of methemoglobin values was wide in all groups, and experimental animals did not differ significantly from controls. Means and standard deviations for the percentages of methemoglobin

Table 1. Hematological values for gulls and puffins receiving daily oral doses of a Prudhoe Bay crude oil. Blood was drawn 96 hours after the birds had received their first dose (mean ± standard deviation). The values for extractable fluorescence (EF) are expressed as arbitrary fluorescence units at a "sensitivity" setting of 5; PCV, packed cell volume; GSH, reduced glutathione; ND, not determined.

Crude oil treatment (ml kg^{-1} day^{-1})	N	PCV (%)	Plasma total solids (g protein/dl)	Hemoglobin g/dl)	Reticulocyte count (%)	Heinz-body count (%)	$\frac{GSH}{PCV}$ (mg/ml)	EF
				Herring gulls (experiment 1)				
Controls	7	30.3 ± 4.5	4.2 ± 0.3	9.2 ± 1.3	7.2 ± 3.1	0.3 ± 0.5	2.60 ± 0.37	ND
10	7	16.9 ± 5.1†	4.0 ± 0.1	5.0 ± 0.8†	26.2 ± 8.2†	92 ± 7†	3.54 ± 0.48†	ND
20	6	14.7 ± 4.4†	4.5 ± 0.2	6.0 ± 1.4†	26.2 ± 8.0†	90 ± 6†	3.52 ± 0.36†	ND
				Herring gulls (experiment 2)				
Controls	12	30.8 ± 1.9	4.9 ± 0.7	8.7 ± 0.6	7.9 ± 2.0	0	2.78 ± 0.30	22.3 ± 11.8
1	12	30.2 ± 2.4	4.8 ± 0.4	8.6 ± 1.0	7.7 ± 2.9	0	2.87 ± 0.32	31.6 ± 19.1
4	12	28.7 ± 2.8*	4.6 ± 0.5	8.2 ± 1.3	7.2 ± 2.9	0	3.02 ± 0.34	26.5 ± 4.4
				Atlantic puffins				
Controls	7	42.1 ± 3.5	5.1 ± 0.3	12.1 ± 1.0	3.9 ± 1.3	0.1 ± 0.2	2.39 ± 0.22	42.2 ± 3.3
5	7	45.1 ± 7.2	4.8 ± 0.6	12.4 ± 2.0	2.9 ± 1.9	0	2.29 ± 0.23	62.8 ± 10.9†
10	5§	28.6 ± 7.5*	5.6 ± 1.2	7.4 ± 1.7†	8.5 ± 1.7†	50 ± 29‡	2.58 ± 0.17	118.7 ± 22.3†

Significant differences from respective control means are indicated as $*P \leq .05$, $\dagger P \leq .01$ (Student's t-test), and $\ddagger P \leq .05$ (Mann-Whitney U test). §One bird died before sampling.

were as follows: control group, 3.08 ± 2.92; group receiving 10 ml kg^{-1} day^{-1}, 6.79 ± 4.40; and group receiving 20 ml kg^{-1} day^{-1}, 4.65 ± 1.56. The concentration of GSH was expressed in milligrams per milliliter of packed red cells for comparison between anemic and control birds; GSH/PCV was elevated in anemic birds (Table 1).

In experiment 2, the same procedure was used to test doses of 1 and 4 ml of PBCO per kilogram per day. Herring gulls receiving the high dose differed slightly from controls only in PCV (Table 1).

To determine the timing of the anemic change, two groups of 20 birds each were established as experimental and control groups, and ten birds from each group were bled on alternate days, beginning 10 hours after the first dose. Oil-dosed birds received 10 ml of PBCO per kilogram per day, and controls received empty gelatin capsules. The PCV and hemoglobin fell precipitously in oil-dosed birds 4 to 5 days after they had received the first dose (Fig. 2). The appearance of Heinz bodies in red cells, a significant increase in GSH/PCV, and a marked regenerative response were coincident with the anemic change.

Alcids (auks) are strictly marine species that commonly are contaminated after oil spills (2). To determine the susceptibility of a representative alcid to the anemia of oil ingestion, 20 nestling puffins weighing 125 to 250 g each were captured on Great Island and placed in artificial burrows prepared near the laboratory. They were fed capelin and maintained for 10 days to ensure the adequacy of the housing and feeding technique. All were gaining weight and feathering at the end of this period. Six or seven birds were assigned to each of three experimental groups and given either 5 or 10 ml of PBCO per kilogram per day or empty gelatin capsules. We monitored the timing of anemic changes by taking 200-μl blood samples 48 and 96 hours after starting the experiment. The PCV decreased after 96 hours. Larger blood samples were taken, and the birds were killed and necropsied at that time. The hematological changes were similar to those seen in herring gull nestlings (Table 1). A graded response to dose was evident only for EF. There was no evidence of external or internal hemorrhage at necropsy.

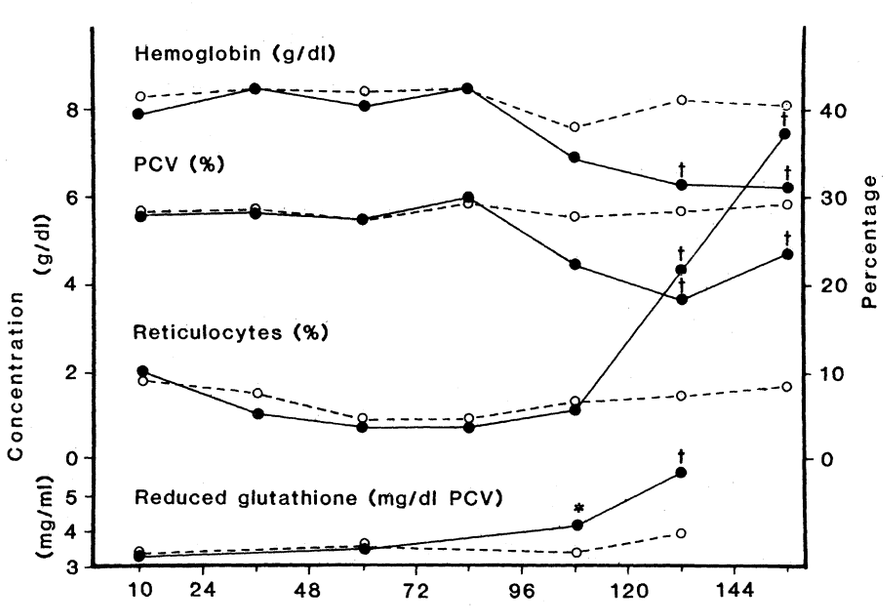

Fig. 2. Hematological values for herring gull nestlings receiving daily oral doses of 10 ml of Prudhoe Bay crude oil per kilogram per day (solid lines) or empty gelatin capsules (broken lines); PCV, packed cell volume. Significant differences between means are indicated as $*P \leq .05$ and $\dagger P \leq .01$ (Student's t-test).

In these experiments, severe hemolytic anemias occurred in two species of marine birds ingesting large amounts of one crude oil. The target tissue was the circulating red cell, and the toxic mechanism involved oxidative changes. The EF data must be interpreted with caution. The PBCO contains fluorescent compounds, and the EF may reflect the absorption of these (8). The value of GSH/PCV was consistently elevated in anemic gulls. This was not expected, since depletion of GSH is commonly associated with oxidant damage in mammalian cells. The influx of reticulocytes, which may contain more GSH than mature red cells, could have contributed to the rise in GSH/PCV, but GSH/PCV was

significantly elevated prior to an increase in reticulocytes (Fig. 2). The correlation coefficients for GSH/PCV and reticulocyte count for each group on each sample day were not significant when compared with published critical values (*13*). Elevated GSH/PCV may indicate a unique biochemical response by avian red cells experiencing oxidant stress or a toxic mechanism involving reduced activity of a GSH-dependent enzyme such as glutathione peroxidase. More detailed biochemical studies are needed before these GSH data can be interpreted. Anemia was reported in earlier experimental studies of oil ingestion by birds, but it was not characterized and generally was not considered significant (*5, 14*).

The amount of oil ingested by wild birds that become oiled is not known, and thus we cannot precisely evaluate the environmental implications of our experimental results. Subtle but significant changes in red cell life-span or function may occur at doses that do not produce overt anemia. Disturbances of red blood cell function potentially can affect many other body tissues. Pathological changes described in studies of oil ingestion by birds (*4–6*) may be, in some cases, secondary or tertiary responses to a primary dysfunction of the erythron.

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8. An analysis of the PBCO used in this study was reported in the study of Peakall *et al.* (*6*).
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Climatic Effects of Atmospheric Carbon Dioxide

Hansen *et al.* (*1*) have used numerical models to provide some insight into why and how the climate will respond to increasing CO_2 concentrations. In addition, however, they argue that the consistency of results from one-dimensional climate models and from observations of global surface air temperature over the last 100 years indicates that the climate is warming due to increasing CO_2 concentrations as global models predict. I agree that the climatic record is not inconsistent with the projected warming to be expected if there is to be an increase of 2 to 3 K for a doubling of CO_2 concentrations and strongly agree that first detection of such changes should be sought by analyses such as done by Hansen *et al.* However, there are a number of limitations in their analysis that must be resolved if we are to say with as much confidence as their article conveys that the initial climatic response to increasing CO_2 has been detected. Among the issues to be resolved are the following.

Although observations [such as figure 3 in (*1*)] show a global cooling from the late 1930's to the early 1960's, the results of Hansen *et al.* with their best model show a much smaller decrease. Thus, while their curve looks good, it chops off the peaks and valleys over the last 60 years. Is that because of natural fluctuations or because of a serious omission in the model? We do not yet know.

Even the very small decrease in temperature from 1930 to 1960 shown by Hansen's model is strongly dependent on the physically untested postulation of Hoyt (*2*) concerning umbra/penumbra ratio. Hansen *et al.* comment that Hoyt's hypothesis was the only one of three viable contenders concerning solar activity that worked. This aspect of their work is extremely uncertain. In addition, their analysis does not consistently apply in each of their three areas. For example, the 1935 to 1960 cooling takes place almost exclusively north of 23.6°N. They do not explain why umbra/penumbra only works in that region (in an exaggerated way) and not over the other 70 percent of the globe.

Hansen *et al.* have not analyzed the volcanic results on a hemispheric or regional basis. Why, for example, does the near-equatorial Mount Agung eruption in 1963 have a larger effect in the Northern than the Southern Hemisphere when observations show much more aerosol in the Southern Hemisphere? The answer usually given is that the ocean's thermal inertia is larger in the Southern Hemisphere, but in some other cases—for example, around 1900 to 1910—the response was much larger in the Southern Hemisphere, yet many of the volcanic eruptions at that time were in the Northern Hemisphere.

The authors indicate that their results tend to confirm model results for global climate change. Virtually all of these