

References and Notes

1. M. Lindauer and H. Martin, in *Animal Orientation and Navigation*, S. R. Galler, L. Schmidt-Koenig, G. J. Jacobs, R. E. Belleville, Eds. (NASA Publ. SP-262, National Aeronautics and Space Administration, Washington, D.C., 1970), p. 559.
2. W. T. Keeton, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 102 (1971); W. E. Southern, *Wilson Bull.* **86**, 256 (1974); C. Walcott and R. P. Green, *Science* **184**, 180 (1974); S. T. Emlen, in *Avian Biology*, D. S. Farner and J. R. King, Eds. (Academic Press, New York, 1975), vol. 5, p. 129; W. Wiltschko, N. J. Demong, R. Wiltschko, S. Bergman, *Science* **193**, 505 (1976).
3. The sensitivity of birds to small atmospheric changes [see M. L. Kreithen and W. T. Keeton, *J. Comp. Physiol.* **89**, 73 (1974)] and polarization of light (*ibid.*, p. 83) was demonstrated with a classical conditioning technique; sensitivity to magnetic stimuli could not be demonstrated in a sample consisting of many birds known to be disoriented by small magnets in homing experiments [*ibid.* **91**, 355 (1974)].
4. W. E. Southern, *Science* **189**, 143 (1975).
5. Then called Project Sanguine. The facility is located in the Chequamegon National Forest in Wisconsin and is a test system for a much larger operational system being proposed for northern Michigan.
6. Our study was part of a larger investigation conducted by T. C. Williams, whose more extensive body of data has not yet been analyzed. Details of methods, negative and ancillary results, and a summary of Williams' observations can be found in T. C. Williams and J. M. Williams, "Final report" (contract N00014-75-00341, U.S. Navy Office of Scientific Research, Arlington, Va., 1976).
7. The antenna wave form was reported to have no d-c component and to be frequency-modulated (FM) in a digital fashion (minimum shift keying) between 72 and 80 hertz. The modulation occurred instantaneously and in phase so that no intermediate frequencies were generated. At all times when the antenna was in condition "on," it was transmitting a test FM signal designed for long-distance communication.
8. Field intensities are estimates based on the assumption that the legs of the antenna are infinite straight-line segments. We requested measurements of the intensities in air above the antenna system, but these measurements were not made.
9. Early in the experiment, the antenna current was changed regularly every 15 minutes in a pseudorandom fashion and the maximum current was nominally 300 amp. Later, the current was sometimes held to a nominal maximum of 150 amp and the experimenters requested a change in antenna condition while radar tracking was in progress. No difference in the proportion of reactions to antenna state was observed between the two protocols, and therefore the data were pooled.
10. The Δ condition always designated a transition from 0 to 75 amp or from 75 to 0 amp within a radar track. No description of the wave form generated by such a transition was available. Those tracks ($N = 57$) which did not include such a transition but which did include at least a portion of the ramping change were omitted from the analysis. Seven of the 57 (12 percent) showed nonlinearities. Without introducing assumptions regarding the latency of reaction and the nature of an effective magnetic stimulus, it was impossible to classify such cases.
11. The radar is type AN/MPQ-29, wavelength 3 cm, peak power 40 kw, pulse length 0.25 μ sec, pulse repetition rate 3800 hertz, vertical polarization, beamwidth 3°, and nutating scan at about 30 hertz. It has been used in studies of the reactions of birds to lights and aircraft.
12. See R. P. Larkin, J. R. Torre-Bueno, D. R. Griffin, C. Walcott, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1994 (1975).
13. Artifacts were generated by the electronic interface which produced voltages proportional to antenna position and range. They were easy to identify and are discussed more fully in the Navy report (6).
14. E. Batschelet, *Am. Inst. Biol. Sci. Monogr.* **1** (1965).
15. For each night, differences in mean directions and speeds between any two steady antenna conditions, including at least five birds each, never exceeded the sum of the two standard deviations.
16. We suspect that gusts of wind caused some XY nonlinearities at low altitudes.
17. Because we did not anticipate that birds would react to current changes in the antenna, there was a ± 1 -minute uncertainty in timing the antenna current change for the first four nights of data collection. The timing error was approximately ± 3 seconds for the other seven nights.
18. The choice of specific "on" condition (NS, EW, or NS+EW) did not seem to affect the frequency or the nature of the nonlinearities. No pattern of change in antenna condition (for example, NS+EW to NS, or NS+EW to EW), initial condition, or final condition was found. On the basis of a paired-comparison analysis, we concluded that there was no significant tendency for birds exhibiting nonlinearities to be closer to an energized antenna than controls at the same point in time relative to the beginning of the track (Wilcoxon matched pairs signed ranks test, $P \geq .05$). Comparing data for individual nights, we found no relationship between the 3-hour K values (an index of natural magnetic activity) and either the presence of nonlinearities, the standard deviations of the flight directions, or the mean flight directions. Values of Kp (a global index of K) ranged from 0+ to 4+. Data were obtained from the World Data Center A, Boulder, Colo. Furthermore, on the basis of a visual examination of the computer plots, we concluded that the presence or geometrical configuration of a nonlinearity was not influenced by the position or path of a bird, nor was the configuration of a nonlinearity influenced by the antenna condition.
19. W. T. Keeton, T. S. Larkin, D. M. Windsor, *J. Comp. Physiol.* **95**, 95 (1974).
20. W. E. Southern, *Condor* **74**, 102 (1972).
21. We thank S. Torre-Bueno and D. Thompson for assistance with the analysis of data; P. Bergschneider, S. Kasieta, and J. Sperry for cooperation in operating the WTF; and D. R. Griffin and T. C. Williams for advice and comments on the manuscript. The radar facility is supported by NSF grant BNS 74-07693 and by a grant from the Mary Flagler Cary Charitable Trust. Expenses for travel and supplies were borne by U.S. Navy grant N00014-00341 to T. C. Williams and J. M. Williams, without whose invitation and participation the project would not have been possible.

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Altered Yolk Structure and Reduced Hatchability of Eggs from Birds Fed Single Doses of Petroleum Oils

Abstract. *Yolk deposited by Japanese quail was abnormal for 24 hours after the oral administration of a single capsule containing 200 milligrams of bunker C oil. Both the structure and the staining properties of the yolk were affected. Fewer eggs were laid during the 4 days after dosing, compared to controls, and hatchability was drastically reduced. Hatchability returned to normal in 4 days. Three other reference oils also affected yolk structure. Canada geese given 2 grams and chickens given 500 milligrams of bunker C oil produced eggs with abnormal yolk rings.*

The devastating effects of massive oil spills on seabird survival are well documented (1), but little is known about the effects of exposure to oil on bird reproduction. Others have reported that the coating of eggs in nests by spraying (2) or by contact from oiled feathers (3) reduced hatching, presumably by interfering with normal respiratory gas exchange through the shell.

We now report that yolk structure, egg production, and hatchability were affected by feeding single doses of bunker C oil, a high-viscosity fuel oil, to Japanese quail (*Coturnix coturnix japonica*). Structural effects on yolk were also noted with bunker C oil in chickens (*Gallus gallus domesticus*) and in Canada geese (*Branta canadensis moffitti*), but effects on hatchability were not tested with these species. Two crude oils and a high aromatic No. 2 fuel oil also affected yolk structure in quail and chickens. The birds most vulnerable to the hazards of oil ingestion are seabirds and waterfowl (1). However, we used quail as an available model animal to simulate the exposure that a wild female bird would experience if she ingested a small amount of oil during yolk formation.

Young laying quail 10 to 24 weeks of age, weighing 130 to 150 g, were maintained as mated pairs in cages, at 21° to 23°C with 14 hours of light commencing at 7:30 a.m. Water and food were freely

available. The diet was a commercial turkey starter mash containing 26 percent crude protein supplemented with small pieces of oystershell. Laying chickens used in another experiment were fed a 16 percent protein diet. Canada geese were fed a 24 percent protein turkey breeder diet. Eggs were studied by freezing, fixing, and staining to reveal structural variation (4). They were degassed under vacuum overnight, frozen in air at -20°C for 24 hours, thawed briefly in water to remove the shells, and fixed in 4 percent formalin at 65°C for 18 hours. They were then cut in half and one half was put into 6 percent aqueous potassium dichromate for 16 hours at 65°C, washed to destain, and sliced at a thickness of 2 mm. Slices from the formalin-fixed half were stained with acidified potassium ferrocyanide to reveal available iron (4). Most of the work was done with a Venezuelan bunker C oil, one of the four reference oils of the American Petroleum Institute (5). The other three were a Kuwait crude oil, a south Louisiana crude oil, and a high aromatic (approximately 40 percent) No. 2 fuel oil. Weighed amounts of oil were fed in capsules (capsule No. 3 for quail; No. 000 for chickens and geese). Oils used as controls were a mineral oil (light, National Formulary, Pilgrim) and a refined safflower seed oil (Saffola). For the hatchability trials, the females of mated

pairs of quail were given capsules containing oil, or empty capsules as controls. Eggs were set weekly, and infertile eggs or dead embryos were removed at 7 days and 14 days. The hatching time limit was set at 18 days.

Several trial experiments were performed with quail to determine the amounts of oil that were effective in reducing egg production and altering yolk structure. There was no mortality of adults in any experiments. Egg production was halted for 6 to 8 days by a 500-mg dose of No. 2 fuel oil; 500 mg of bunker C oil was more toxic, halting egg production for the duration of the 2-week trial. A dose of 200 mg of bunker C oil caused a reduction in egg production, but 100 mg had no apparent effect on production. Neither mineral oil (500 mg) nor safflower seed oil (500 mg) reduced egg production. Yolk structure, which was distinctly affected by all four reference oils, was clearly different from control eggs laid by birds fed mineral or vegetable oils.

After the trials to determine tolerance levels, four production and hatchability experiments were performed over a period of 9 weeks. Experiments 1, 2, and 3 utilized one group of quail. In experiment 1, 18 female quail were each given 100 mg of bunker C oil between 9:00 and 9:30 a.m.; and 19 were each given an empty capsule. In confirmation of the previous experiments, egg production was not reduced significantly (by the chi-

square test), and there was no significant decrease in hatchability of the eggs laid after the bunker C oil was given. In experiment 2 there were 20 females (ten from each group of experiment 1) which were given 200 mg of bunker C oil, and 19 control females (the remaining 17 from experiment 1 plus two others) which were given empty capsules. Production and hatchability were allowed to return to normal for 14 days before experiment 3 was begun. In experiment 3, in order to compensate for possible cumulative effects of dosing, the female quail from experiment 2 and additional untreated birds were randomly divided into a group of 48 birds given 200 mg of bunker C oil and a group of 19 birds given empty capsules. The results of experiments 2 and 3, completed over a total of 33 days, were very similar, and are combined in Fig. 1. Egg production was reduced on days 1 and 2 ($P < .001$) following the oil dose. Hatchability was reduced markedly on days 1 and 2 ($P < .001$) but returned to normal by day 4.

When 200 mg of bunker C oil was given, the effects on egg production and hatchability were transient. Cumulative effects were not observed; however, the experiments were not designed to evaluate this possibility.

Quail kept on a simulated daylight schedule normally deposit two different rings of yolk in a 24-hour period; yolk deposited during the light hours becomes a dark ring when stained with dichromate, while that deposited during the night forms a light-staining ring (4). Usually, 4 to 6 days are required for the rapid stage of yolk formation in quail (6), and after ovulation approximately 24 hours are required for formation of white, membranes, and shell (7). Oviposition usually occurs during the last 6 hours of the light period (8). Chickens differ from quail in that 7 to 11 days are usually required for yolk formation (9), and most eggs are laid during the first 6 hours of the light period (10).

Yolk that was deposited by quail after the administration of 200 mg of bunker C oil exhibited several abnormalities, as shown in Fig. 2. Typically, yolk deposition became uneven during the first few hours after oil dosage. Less than the normal amount of yolk was deposited during the first night, forming a thin layer of yolk that did not take up the dichromate stain. The yolk spheres in this layer were very small (10 to 30 μm in diameter) in contrast to the normal size of 40 to 120 μm (11). The next outer yolk ring was narrow and abnormally dark when stained by dichromate. In some eggs the yolk spheres became separated during

fixation, and cracks developed in the yolk ring. Lightly staining yolk was also deposited during the second night, followed by another dark ring the next morning. Eggs from quail given a dose of 100 mg of bunker C oil showed only one abnormal ring. Generally similar patterns were observed in birds given the two crude oils and the No. 2 fuel oil, but the levels required to produce the effects were higher for the crude oils. With bunker C oil, but not the other oils, a characteristic ring of yolk outside the first narrow ring was found to stain darkly with acidic ferrocyanide in the Prussian blue test for available iron (4). Iron analyses have not yet been done, and we have no explanation for this effect.

In addition to the observed effects on yolk structure, oil ingestion was often followed by formation of thin shells that were easily cracked. Some shells were af-

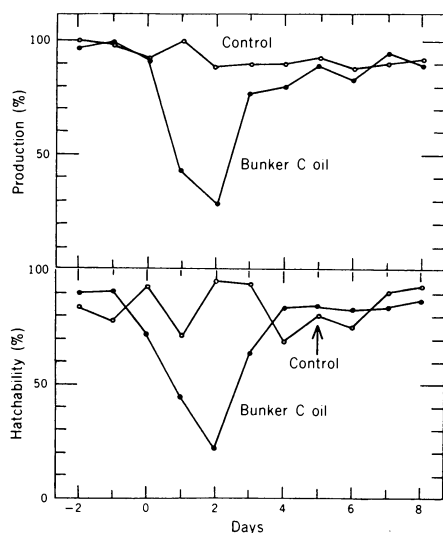


Fig. 1. Egg production and hatchability in quail given a single dose of 200 mg of bunker C oil at 9:00 a.m. of day 0. Eggs are normally laid between 6:00 and 10:00 p.m. Production is expressed as eggs laid per day per 100 quail hens, hatchability as percentage of fertile eggs hatched (fertility determined after 7 days of incubation). Fertility and early deaths were independent of treatment. There were 68 experimental and 38 control females.

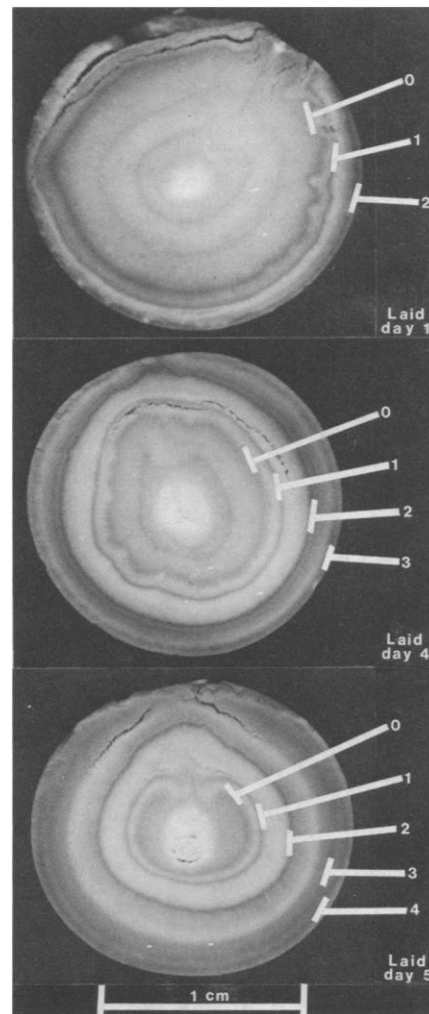


Fig. 2. Sections of fixed and dichromate-stained yolks from a quail that was fed a single dose of 200 mg of bunker C oil on day 0. The eggs were laid 3, 4, or 5 days after dosage. The normal ring structure for this bird is shown by the inner rings of the first egg. Cracks in the yolk rings are common effects of oil dosage.

fects the day that the oil was given.

When single doses of the four test oils were given to chickens, the effects on yolk structure were similar to those observed in quail. For example, 500 mg of bunker C produced a marked structural effect on the yolk; 3 g caused cessation of egg production. In some chicken eggs, the yolk deposited 2 or 3 days after oil dosage was abnormally dark when stained by dichromate. The characteristic Prussian blue line mentioned above was associated with the bunker C dosage, but not the other oils.

Two Canada geese (*Branta canadensis moffitti*) which were kept in a large outdoor pen were dosed with 2, 3, or 5 g of bunker C oil at various times during yolk formation, and the eggs were collected, frozen, fixed, and stained. Eggs laid after dosing showed a characteristic light ring after dichromate staining; yolk deposited later stained darkly.

The mechanisms of oil action on avian reproduction are not known. It is possible that toxic components of oils are absorbed from the intestinal tract and transported by the plasma to the liver and thence to the ovary, where they may be deposited in the yolk, as are some carotenoids (12). There may also be less direct effects. For example, petroleum products similar to those used in the present experiments were found to inhibit sodium and water absorption by the intestinal mucosa of ducklings given hypertonic saline solutions (13). Purified mineral oils had little effect. Yolk contains approximately equal amounts of sodium and potassium, in contrast to the sodium-rich plasma from which yolk is derived (14); hence, disturbances in sodium and potassium metabolism might be expected to influence yolk formation and embryo survival.

The effects of oil on yolk structure are easily differentiated from the effects of other environmental variables that we have studied, including food deprivation for periods up to 24 hours, water deprivation for 12 hours, and calcium deficiency.

Observations of changes in yolk formation such as we have described may be useful in monitoring oil pollution of breeding populations of wild birds.

The birds most at risk from oil pollution, primarily seabirds and waterfowl, have many features in common with the quail, but direct studies of wild birds have yet to be made.

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References and Notes

1. A. Nelson-Smith, *Oil Pollution and Marine Ecology* (Elek, London, 1972); D. C. Smith, in *1975 Conference on Prevention and Control of Oil Pollution, San Francisco* (American Petroleum Institute, Washington, D.C., 1975), p. 241.
2. U. K. Abbott, R. M. Craig, J. O. Keith, *Poult. Sci.* **43**, 1297 (1964). It was found in this study of pesticides sprayed onto nest and eggs that the carrier oil was damaging.
3. R. Hartung, *J. Wildl. Manage.* **29**, 872 (1965).
4. C. R. Grau, *Poult. Sci.* **55**, 1418 (1976).
5. The API reference oils were obtained from J. W. Anderson, Texas A & M University, College Station. Chemical compositions were determined by S. Warner, Battelle Memorial Laboratories, Columbus, Ohio. Supplementary data were supplied by R. J. Pancirov, Esso Research and Engineering Company, Linden, N.J. For a description of samples, see *API Report No. AID IBA. 74* (American Petroleum Institute, Washington, D.C., 1974).
6. K. Homma, W. O. Wilson, L. Z. McFarland, *Am. Zool.* **5**, 185 (1965); W. L. Bacon and M. Koontz, *Poult. Sci.* **50**, 233 (1971).
7. A. E. Woodard and F. B. Mather, *Poult. Sci.* **43**, 1427 (1964).
8. L. C. Arrington, H. Abplanalp, W. O. Wilson, *Br. Poult. Sci.* **3**, 105 (1962).
9. A. B. Gilbert, in *Egg Formation and Production*, B. M. Freeman and P. E. Lake, Eds. (British Poultry Science, Edinburgh, 1972), p. 3.
10. A. B. Gilbert and D. G. M. Wood-Gush, in *Physiology and Biochemistry of Domestic Fowl*, D. J. Bell and B. M. Freeman, Eds. (Academic Press, London, 1972), vol. 3, chap. 57, p. 1353.
11. R. Bellairs, in *Advances in Morphogenesis*, M. Abercrombie and J. Brachet, Eds. (Academic Press, New York, 1964), vol. 4, p. 217.
12. N. P. Williams, R. E. Davies, J. R. Couch, *Poult. Sci.* **42**, 691 (1963).
13. A. D. Crocker, J. Cronshaw, W. N. Holmes, *Environ. Physiol. Biochem.* **5**, 92 (1975).
14. A. H. Smith, W. O. Wilson, J. G. Brown, *Poult. Sci.* **33**, 898 (1954).

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Rapid Brain Cooling in Exercising Dogs

Abstract. *In alert, resting dogs, the brain is warmer than arterial blood in the common carotid artery. When dogs run, brain temperature drops, despite a sharp rise in carotid blood temperature, and is maintained 1.3°C below carotid temperature during exercise. This brain cooling apparently results from countercurrent heat exchange between warm arterial blood supplying the brain and cool venous blood draining the nose and mouth. The heat exchange occurs in the arteries at the base of the brain, which form a rudimentary carotid rete in the dog, and is greatest during exercise, when respiratory evaporation is at a peak. In animals with a carotid rete, the brain is protected against overheating during the severe thermal stress of exercise.*

It has been known for almost 10 years that some of the carnivores and hoofed mammals have an intracranial vascular heat exchanger in which arterial blood destined for the brain can be cooled (1). The heat exchanger is a plexus of small arteries, called the carotid rete, which is surrounded by venous blood that drains the nasal and oral passages (2). Warm blood in the arterial plexus loses heat to venous blood, which has been cooled by evaporation in the nose and mouth. The mammals with a carotid rete are panting animals. When they are panting, evaporation from the nose and mouth is increased and the brain is kept cooler than other deep body regions (1). Since the brain is sensitive to overheating, the carotid rete provides an extra measure of heat tolerance in those mammals in which it is present. In contrast, in animals without a rete, the temperature of blood supplying the brain is the same as temperature of blood in the body core, even during heat stress (3).

The position of the dog in this schema has been puzzling, for the dog has only a rudimentary rete (2). Under some conditions, the blood supplying the brain of the dog has been observed to cool below the temperature of blood in the body core, but the degree of cooling is small compared to the cooling present in animals with well-developed carotid retia

(4). For example, in cats and sheep panting in hot, dry environments (45° to 50°C), cerebral arterial blood in the carotid rete is cooled more than 1°C below the temperature of blood in the body core (1). In dogs exposed to hot, dry air, the blood supplying the brain is cooled less than 0.50°C below core blood temperature (4). It was assumed that it was not possible for dogs to achieve significant brain cooling during thermal stress because the surface area of the carotid rete heat exchanger is smaller in the dog than in these other animals. Yet dogs are renowned for their high heat tolerance and especially for their capacity to perform physical work in the heat for long periods of time (5). We now report that during heavy exercise in a warm environment, the brain of the dog cools more than 1.3°C below the temperature of blood leaving the heart. It seems that maximum cooling of the brain in animals with a carotid rete occurs during exercise.

We trained two large mongrel dogs (30 and 35 kg) to run on a motor-driven treadmill at a speed of 7.2 km/hour and a slope of 14 percent. Even though they could run at this speed at a fast trot, the treadmill slope increased the severity of the exercise and neither dog could run longer than about 20 minutes. After a training period of 2 weeks, each animal was