fected 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.

C. R. Grau, T. Roudybush
J. Dobbs, J. Wathen
Department of Avian Sciences,
University of California,
Davis 95616

25 FEBRUARY 1977

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).

10 August 1976; revised 24 September 1976

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.*

In 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

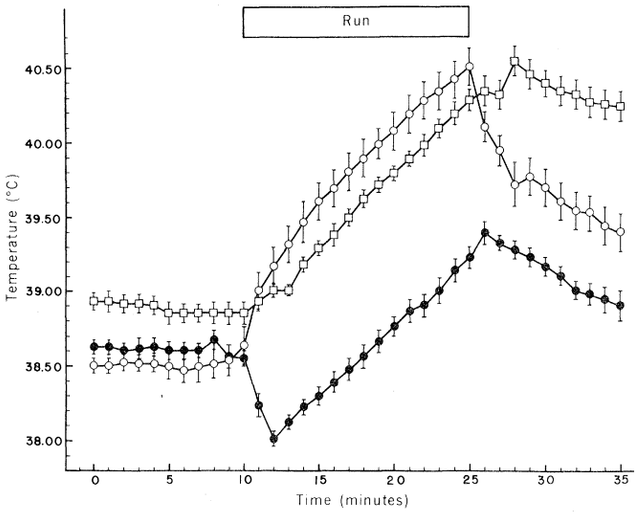

Fig. 1. Brain (●), carotid blood (○), and rectal (□) temperatures during exercise in dogs. Points are mean temperatures calculated each minute from eight experiments, four experiments on each of two dogs. Vertical lines are standard errors. Between minutes 0 and 10, the dogs were at rest on the treadmill, usually lying down. During the period marked *Run* (minutes 10 to 25) the dogs ran at a speed of 7.2 km/hour up a slope of 14 percent. At minute 25, the treadmill was stopped and the animals sat on the treadmill for the 10-minute recovery period. Ambient temperature was 30°C.

anesthetized and copper-constantan thermocouples were implanted in the hypothalamus near the cerebral arteries and in the common carotid artery through one of its thyroid branches in the neck. Thermocouple leads were attached to plugs cemented to the skull (*1*). In all mammalian species studied, hypothalamic temperature is slightly higher (0.05° to 0.30°C) than cerebral arterial blood temperature, and it rapidly reflects changes in cerebral arterial temperature. Structures in the middle of the brain, farther from the source of arterial blood, are warmer (*1, 3, 4*). When the animals had completely recovered from surgery, we measured their brain, carotid blood, and rectal temperatures while they ran on the treadmill for 15 minutes in a warm room (30°C). Each dog was studied during four separate running experiments. Thermocouple outputs were recorded continuously on an ink-writing polygraph with an accuracy of 0.05°C. Temperatures were calculated from the polygraph records at 1-minute intervals for the 10 minutes before the exercise, the 15-minute exercise period, and the 10 minutes after exercise (Fig. 1).

When the dogs were lying quietly on the treadmill before they began to run, brain temperature was higher than carotid blood temperature. All temperatures were steady until a few minutes before the run, when the dogs became restless, the carotid temperature rose slightly and brain temperature fell slightly. At the onset of exercise, carotid blood temperature rose rapidly and brain temperature dropped. Despite the continued rise in carotid blood temperature, brain temperature fell until minute 3 of exercise and then it began to rise at about the same rate as the carotid temperature. From minute 5 of exercise until the treadmill was stopped, the average brain temperature was maintained about 1.3°C cooler than the carotid blood temperature. At the end of the exercise, carotid temperature fell rapidly while brain temperature continued to rise for the first minute and then fell. Rectal temperature, which rose more slowly than carotid temperature during the run, continued to rise for 3 minutes after the exercise and dropped very slowly during the recovery period.

The degree of brain cooling below carotid blood temperature in these exercising dogs is almost three times as great as brain cooling in dogs at rest in hot, dry environments (*4*). The accelerated brain cooling during exercise probably results from the increase in respiratory ventilation which begins at the very onset of exercise and continues throughout the exercise. Respiratory evaporation in exercising dogs reaches levels double those in panting dogs at rest in hot, dry air (*5, 6*). This increase in respiratory evaporative heat loss during exercise may be due to a change in the pattern of breathing as well as to the increased ventilation rate. Taylor (*6*) has recently observed that exercising dogs breathe in and out through both the mouth and the nose, but panting dogs at rest in the heat breathe in through the nose and out through the mouth. The recruitment of the additional evaporative surface of the mouth during exercise may explain part of the increased evaporation. Venous blood draining both the oral and the nasal passages of the dog can drain into the cranial cavity where it comes into close contact with cerebral arterial blood (*7*). Our data show that the acceleration of respiratory evaporation during exercise, which would lead to maximum cooling of nasal and oral venous blood, allows a high rate of heat exchange in the rudimentary carotid rete of the dog and a significant cooling of the brain.

The advantage for the dog in being able to cool its brain during exercise has been described by calculations made by Taylor (*8*), who showed that for mammals of intermediate size (5 to 200 kg), heavy exercise presents the most severe thermal stress that will ever be encountered. The rate of excess heat production in running dogs is about ten times as great as the highest rate of heat gain that could occur in the hottest desert environments on Earth (*8*). The rate that deep body temperature rises in exercising mammals far exceeds that in mammals at rest in desert environments. Since normal brain functions begin to be disturbed at temperatures only 4° to 5°C above resting temperatures (*9*), cooling the brain more than 1°C below the temperature of blood in the body core could increase an animal's exercise tolerance significantly.

The only other measurements of brain temperature in an exercising animal with a carotid rete were made by Taylor and Lyman (*10*), who found that the Thomson's gazelle cooled its brain 2.7°C below carotid blood temperature while it was running at high speed. Their findings, considered with the present ones on the dog, support the view that, in animals with a carotid rete, higher rates of brain cooling will occur during exercise than at any other time. Animals such as the gazelle, the sheep, and the cat, in which the carotid rete is well developed and provides a large arterial surface area for heat exchange, can probably produce a greater body-brain temperature difference than the dog. However, under conditions of heavy exercise in hot environments, dogs may be capable of greater

brain cooling than we have observed. The ability to keep the brain cool during exercise provides these animals with an advantage over panting animals with no carotid rete. In those animals, such as the rabbit, brain temperature during exercise is higher than the temperature of blood in the body core (*3*). This may underlie, in part, the limited tolerance to heat and to exercise in rabbits and other panting mammals in which brain cooling does not occur.

M. A. BAKER

Program in Biomedical Sciences and *Department of Biology, University of California, Riverside 92502*

L. W. CHAPMAN

Department of Physiology, University of Southern California School of Medicine, Los Angeles 90033

References and Notes

1. For the goat, see C. R. Taylor [*Physiol. Zool.* **39**, 127 (1966)]; for the sheep and for techniques, see M. A. Baker and J. N. Hayward [*J. Physiol. (London)* **198**, 561 (1968)]; for the cat, see M. A. Baker [*ibid.* **220**, 771 (1972)].
2. P. M. Daniel, J. D. K. Dawes, M. M. L. Prichard, *Philos. Trans. R. Soc. London Ser. B* **237**, 173 (1953).
3. For the rabbit, see M. A. Baker and J. N. Hayward [*Science* **157**, 1586 (1967)] and also M. A. Baker and M. J. Cronin [*Fed. Proc. Fed. Am. Soc. Exp. Biol.* **35**, 481 (1976)]; for the monkey, see J. N. Hayward and M. A. Baker [*Am. J. Physiol.* **215**, 389 (1968)].
4. J. H. Magilton and C. S. Swift, *Physiologist* **10**, 241 (1967); J. N. Hayward, *Exp. Neurol.* **21**, 201 (1968); M. A. Baker, L. W. Chapman, M. Nathanson, *Respir. Physiol.* **22**, 325 (1974).
5. K. Schmidt-Nielsen, *Desert Animals: Physiological Problems of Heat and Water* (Oxford, New York, 1964); C. R. Taylor, K. Schmidt-Nielsen, R. Dmi'el, M. Fedak, *Am. J. Physiol.* **220**, 823 (1971).
6. C. R. Taylor, in *International Review of Physiology, Environmental Physiology II*, D. Robertshaw, Ed. (University Park Press, Baltimore, in press).
7. M. E. Miller, G. C. Christensen, H. E. Evans, *Anatomy of the Dog* (Saunders, Philadelphia, 1964). There are extensive interconnections among the veins draining the face, the nasal passages, and the oral passages. Oral and nasal venous blood entering the deep facial vein can flow either into the external jugular system or into the cranial cavity through the pterygoid plexus or the ophthalmic veins, respectively. Circular smooth muscle in some of these veins may allow blood to be shunted toward one or the other pathway [J. H. Magilton and C. S. Swift, *J. Appl. Physiol.* **27**, 18 (1969)].
8. C. R. Taylor, in *International Review of Physiology*, vol. 7, *Environmental Physiology*, D. Robertshaw, Ed. (University Park Press, Baltimore, 1974), p. 163.
9. N. Malamud, W. Haymaker, R. P. Custer, *Mil. Surg.* **99**, 397 (1946); D. Minard and L. Copman in *Temperature, Its Measurement and Control in Science and Industry*, vol. 3, *Biology and Medicine*, J. D. Hardy, Ed. (Reinhold, New York, 1963), p. 253; S. Shibolet, M. C. Lancaster, Y. Danon, *Aviat. Space Environ. Med.* **47**, 280 (1976).
10. C. R. Taylor and C. P. Lyman, *Am. J. Physiol.* **222**, 114 (1972).
11. We thank Dr. C. R. Taylor for suggesting these experiments. Supported by NSF grant BMS 74-14665.

4 October 1976; revised 12 November 1976

Hereditary Hemolytic Anemia with Increased Red Cell Adenosine Deaminase (45- to 70-fold) and Decreased Adenosine Triphosphate

Abstract. *Hereditary hemolytic anemia, a dominantly transmitted disorder, has affected 12 family members spanning three generations. The concentration of adenosine triphosphate in the red cells was about half that of comparably reticulocyte-rich blood. Since adenosine deaminase and adenosine kinase compete for a common substrate, the greatly increased activity of the former may interfere with nucleotide salvage via the latter.*

In 1970, we briefly reported a kindred in which dominantly transmitted hereditary hemolytic anemia associated with marked reduction in erythrocyte adenine nucleotides was present in several members (*1*). In the proband, the syndrome was characterized by nonspherocytic hemolytic anemia, splenomegaly, reticulocytosis up to 22 percent, a negative Coombs' test for autoimmune hemolytic anemia, and no evidence of hemoglobinopathy. The activities of erythrocyte enzymes of the Embden-Meyerhof pathway, of the dehydrogenases of the hexosemonophosphate shunt, and of adenylate kinase and glutathione (GSH) peroxidase were normal, as was the concentration of GSH. With the exception of nucleotide cofactors, glycolytic intermediates were present in normal concentration. In an expanded investigation of the syndrome to be presented in more detail elsewhere (*2*), we have observed 45- to 70-fold elevations of adenosine deaminase (ADA) activity in the red cells of affected family members.

The proband and 11 of 23 maternal relatives spanning three generations have an identical hemolytic syndrome. The disorder is transmitted as an autosomal dominant, with 4 out of 9 males and 8 out of 13 females at risk affected. The underlying abnormality is intrinsic to the erythrocyte. Labeled proband red cells have a very short ^{51}Cr half-life of 5 days when transfused into the normal father. Conversely, the half-survival of the father's red cells transfused into the proband was a normal 28 days. Anemia is mild and in some instances fully compensated. Reticulocytosis averages 6 percent (range 3.3 to 11.7 percent) and packed cell volumes 38.8 percent (range 35 to 41 percent). Table 1 records red cell nucleotide concentrations (*3*). The appropriate comparison is with comparably reticulocyte-rich blood, since young erythrocytes characteristically contain greater concentrations of nucleotides than do older cells. The adenine nucleotides average about 60 percent of the normal control mean and less than 50 percent of that of reticulocyte-rich blood. No affected subject has levels greater than 75 percent of the normal mean or greater than 50 percent of the mean of controls with reticulocytosis. In contrast, mean adenosine triphosphate (ATP) and total nucleotides in nine unaffected blood relatives are 96 and 103 percent, respectively, of control values.

Ten affected family members thus far studied have red cell ADA activities 45- to 70-fold greater than the normal mean (Table 2). This is confirmed both spectrophotometrically, measuring adenosine conversion to inosine at 265 nm, and by NH_3 released during deamination (*4*). Electrophoretically, proband ADA is of the common ADA 1-1 pattern (*5*), but to reduce gel staining for enzyme activity to normal intensity requires a 40- to 100-fold dilution of hemolyzate. In hemolyzates the apparent K_m (Michaelis constant) for adenosine is normal (20 μM by spectrophotometry, 18 μM by assay of NH_3 production) (*6*). The apparent K_m for adenosine was essentially identical with that of normal ADA 1-1, as was specific activity, heat stability, and K_i (inhibition constant) for the competitive ADA inhibitor guanylurea sulfate (*7*). By all criteria thus far employed, the greatly increased ADA activity represents overproduction of normal enzyme rather than a mutationally altered catalytic protein.

Methylmercaptopurine riboside is a substrate for adenosine kinase but not ADA, and is converted by human red cells to the nucleotide mono-, but not the di- or triphosphate (*8*). On incubation with methylmercaptopurine riboside in the presence of glucose at *p*H 8.0, proband erythrocytes formed large amounts of phosphorylated nucleoside, evidenced by precipitation as the barium salt in 80 percent ethanol at *p*H 8.0. Redissolved in HCl, the washed precipitate exhibited a strong absorbance peak at 296 nm, an indication of the presence of a major component of methylmercaptopurine-containing material. Treatment of the same incubated, neutralized, deproteinized red cell extracts with *Escherichia*