

Brain temperature in the calliope hummingbird (*Stellula calliope*): a species lacking a *rete mirabile ophthalmicum*

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Summary. 1. Hypothalamic and colonic temperature were simultaneously measured in the calliope hummingbird (*Stellula calliope*). The vascular anatomy of the temporal region of these birds was also examined.

2. At air temperatures below 36 °C, calliope hummingbirds regulated their body temperature between 35.6 and 40.6 °C. At higher air temperatures, colonic temperature increased.

3. Brain temperature was lower than body temperature at high colonic temperatures (>39.5 °C). The body-to-brain temperature difference in birds with high body temperatures averaged 0.73 °C and remained relatively constant at all colonic temperatures above 39.5 °C.

4. At body temperatures below 39.5 °C, brain temperature of calliope hummingbirds exceeded colonic temperature by an average of 1.38 °C. The possible explanations for this observation are discussed.

5. Calliope hummingbirds lack a *rete mirabile ophthalmicum*, the countercurrent arterial-venous heat exchanger responsible for brain cooling in other birds.

6. Brain cooling in calliope hummingbirds apparently depends on heat exchange between arterial blood flowing to the brain, and cool venous blood returning from evaporative and convective surfaces at sites other than the *rete mirabile ophthalmicum*.

Introduction

In all birds in which hypothalamic and colonic temperature have been simultaneously measured over a range of air temperatures, brain temperature is below that of the body (see Bech and Midtgård 1981, for a review of the early literature; Pinshow et al. 1982; Arad and Midtgård 1983; Midtgård et al. 1983; Arad et al. 1984; Hudson et al. 1985; Kleinhaus et al. 1985). This body-to-brain temperature difference varies from 0.12 °C in the zebra finch (Bech and Midtgård 1981) to over 2 °C in guineafowl (Crowe and Withers 1979), and is important in birds' exceptional tolerance of high environmental temperatures (Kilgore et al. 1976).

Brain cooling in birds is accomplished by heat exchange between the arterial and venous *retia mirabilia ophthalmica* (collectively the *rete mirabile ophthalmicum* or RMO). In the RMO, arterial blood supplying the brain is cooled by venous blood returning from evaporative and convective surfaces in the buccopharyngeal cavity, beak, nares, and eyes. Evidence for this role of the RMO in lowering brain temperature in birds includes the following: the body-to-brain temperature difference can be eliminated or reversed by blocking blood flow to the RMO (Kilgore et al. 1979) or bypassing the sites of evaporative and convective cooling of venous blood (Bernstein et al. 1979b); the body-to-brain temperature difference is diminished in water deprived and dehydrated birds in which evaporative water loss is generally reduced (Arad and Midtgård 1983; Kleinhaus et al. 1985); the body-to-brain temperature difference can be enhanced by increasing air flow over the corneal cooling surfaces (Pinshow et al. 1982); and there is a temperature gradient in the blood flowing through the RMO (Hudson et al. 1985).

Heat exchange in the RMO and, therefore, the

Abbreviations: RMO *rete mirabile ophthalmicum*; T_{cl} colonic temperature; T_{br} hypothalamic temperature; T_a air temperature

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magnitude of the body-to-brain temperature difference is significantly and positively correlated with the total exchange area of the RMO (Midtgård et al. 1983). Maximal exchange area of the RMO, in turn, is positively correlated with body mass (Midtgård 1983; Midtgård et al. 1983; Arad and Midtgård 1984; Arad et al. 1984). It thus follows that smaller birds generally have reduced heat exchange capacity in the RMO and presumably, would also have a reduced ability to lower hypothalamic temperature. Such is the case in the 13 g zebra finch (Bech and Midtgård 1981).

In a theoretical analysis of heat exchange capability of the RMO, Midtgård (1983), however, found heat exchange capacity to be independent of the complexity of the RMO. Clearly, additional studies of very small birds are needed to clarify the relationship between the anatomical complexity of the RMO and brain cooling in birds. The present study was undertaken to ascertain whether brain temperature is below body temperature in the calliope hummingbird (*Stellula calliope*), a bird with a mass less than 3 g, and to determine if the anatomical complexity of the *rete* reflects such a brain cooling ability.

Materials and methods

Experimental animals. Preliminary experiments were conducted during July and August of 1982 using slightly different methods and procedures, as noted below, from those used in subsequent experiments. The different procedures yielded statistically indistinguishable results so the data have been combined for analysis. Seven female and four male calliope hummingbirds were used in these experiments. The mean (\pm SD) body mass of these eleven birds was 2.5 ± 0.2 g. In captivity, hummingbirds were maintained on a diet consisting of a sugar solution, a protein solution supplemented with vitamins (Biotropic-Verlag GmbH), and live *Drosophila*.

Thermocouples. Welded copper-constantan thermocouples were used to measure brain, colonic, and air temperatures. Thermocouples were constructed from either 0.12 mm (36 AWG) or 0.05 mm (44 AWG) wire (Omega Engineering, Inc.), and were coated with polyvinyl. Colonic probes had a final diameter of 0.2 mm, while brain probes had a final diameter of either 0.1 mm or 0.2 mm. Epoxy cement was used to stiffen those thermocouples constructed from the 0.05 mm wire.

Surgical procedure and thermocouple placement. Thermocouples were implanted in the brain under general anesthesia. Equithesin (2.5 μ l/g; prepared as suggested by Fedde 1978) given intramuscularly was used to anesthetize birds in the preliminary experiments. Subsequently, ketamine hydrochloride (0.1 mg/g; intramuscular) was used. With both anesthetics a medium surgical plane of anesthesia was induced (Altman 1980). During surgery, birds were restrained in a flannel jacket similar to that described by Lasiewski (1962), and a warming tray, set at 40 °C, was used to maintain the birds' body temperature.

The surgical procedure generally followed that used pre-

viously (Kilgore et al. 1973, 1976, 1979, 1981). A sagittal incision was made in the skin off the midline of the skull, the hyoid apparatus was gently retracted, and a hole was made in the skull 0.5 mm behind the posterior border of the orbits and 0.8 mm from the midline using a 22 gauge hypodermic needle. The thermocouple was inserted 2.75 mm into the brain. This placement left the tip positioned 0.5 mm dorsal to the chiasma opticum in the regio preoptica hypothalami. The thermocouple emerging from the skull was bent at a 90° angle, placed flat along the top of the skull, and secured using dental cement. A cement was also used to close the skin. Polyethylene tubing (PE-10) was placed over the wires protruding from the incision to provide extra support. All birds were allowed at least 24 h to recover from surgery before being used in experiments. Thermocouple placement was verified by post mortem examination.

Temperature measurements. All thermocouples were calibrated against a mercury-in-glass thermometer traceable to a National Bureau of Standards calibrated thermometer. The standard errors (imprecision) of temperatures obtained during calibration did not exceed 0.01 °C. The systematic error of temperature measurements also does not exceed 0.01 °C. Imprecision and systematic error are used here as recommended by Eisenhart (1968).

Air, brain, and colonic temperatures were measured simultaneously during an experiment. Air and colonic temperatures were recorded on a multichannel potentiometric recorder (Honeywell, Elektronik 16). The low-voltage output of the brain thermocouple circuit was read directly on a digital multimeter (Keithley, model 160).

Experimental protocol. Birds were maintained in captivity for at least two weeks prior to surgery. All experiments were conducted between 0900 and 1700 h in a darkened constant temperature chamber in which the desired temperature (± 0.1 °C) could be maintained. Because of their size and tendency to enter torpor, birds were not starved prior to experiments. The colonic probe was inserted 0.5 cm into the colon and the brain thermocouple soldered to lead wires. Birds were exposed to air temperatures ranging from 30 °C to 44 °C. During an experiment birds were held at a particular air temperature until brain and colonic temperatures were stable (± 0.1 °C) for 15 min. The air temperature was then raised approximately 4 °C. Individual birds were exposed to between one and seven different air temperatures during an experimental period, with most being exposed to only three. In some instances, individual birds were exposed to only one temperature on a particular day and then were studied again on the following day. These variations in protocol did not have any discernible effect on the results.

Vascular anatomy. The vascular anatomy of six (male and female) calliope hummingbirds was studied bilaterally. The birds were killed by an overdose of anesthetic, the aorta cannulated, and Microfil (Canton BioMedical Supply) injected to facilitate identification of the vessels. The birds were then dissected and the vascular anatomy examined under a dissecting microscope. One head was immersed in 5% sodium hypochlorite solution to remove all soft tissue leaving only bone and injected vessels. Vascular anatomical terminology is based on Baumel (1979).

Statistics. An *F* test was used to test whether a significant portion of the variation in brain temperature was explained by regression on colonic temperature (i.e., if the relationships were truly linear). Nonparametric tests were used to ascertain whether the slopes of the least-squares regression lines were different from unity (Daniel 1978).

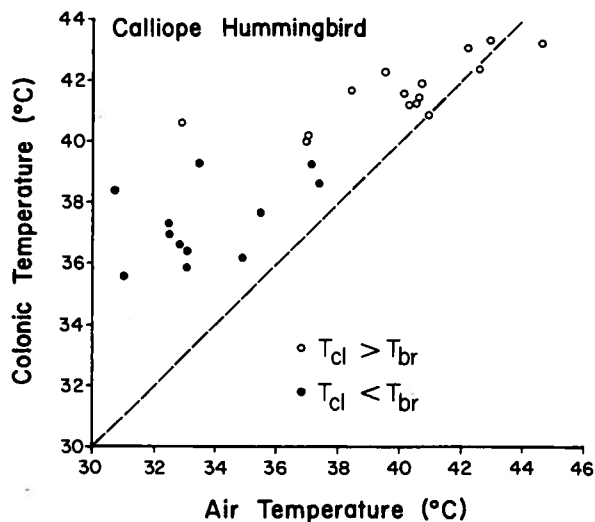


Fig. 1. Relationship between colonic and air temperature in the calliope hummingbird. The dashed line is the isothermal line. Open circles represent measurements when colonic temperature (T_{cl}) exceeded hypothalamic temperature (T_{br}); closed circles represent measurements when T_{br} exceeded T_{cl} .

Results and discussion

Body temperature regulation

At air temperatures below about 36 °C, calliope hummingbirds regulated their colonic temperatures between 35.6 and 40.6 °C without entering torpor, while at higher experimental temperatures T_{cl} increased (Fig. 1). This pattern of body temperature regulation is identical to that reported previously for calliope hummingbirds (Lasiewski 1963) and for other small hummingbirds under similar experimental conditions (Lasiewski 1964; Kruger et al. 1982). Lasiewski (1963), for example, reported that the lowest body temperature of non-torpid calliope hummingbirds in the dark was 35.0 °C.

The highest stable body temperature recorded in these experiments was 43.3 °C.

Body-to-brain temperature difference

Hypothalamic temperature of calliope hummingbirds exceeded colonic temperature at low body temperatures (<39.5 °C), but was lower than colonic at body temperatures above 39.5 °C (Fig. 2).

The relationship between brain and body temperature above colonic temperatures of 39.5 °C is described by the equation:

$$T_{br} = 0.87 T_{cl} + 4.77 \quad (s_{y \cdot x} / \bar{Y} = 0.01, r = 0.92).$$

The slope of this line is not statistically different from unity ($P > 0.05$), but the relationship is linear

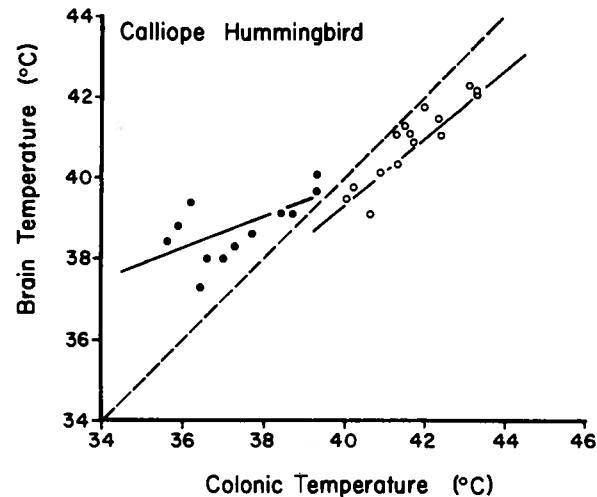


Fig. 2. Relationship between brain and colonic temperature in the calliope hummingbird. The solid lines are the calculated regression lines fitted to these data. The dashed line is the isothermal line. The equations describing the solid regression lines can be found in the text. Symbols as in Fig. 1

($F_{[1,13]} = 70, P < 0.001$). The standard error of the estimate ($s_{y \cdot x}$) has been standardized by dividing it by the mean brain temperature (\bar{Y}). The mean body-to-brain temperature difference at $T_{cl} > 39.5$ °C was 0.73 °C (SD = 0.42; $n = 15$).

This relationship between hypothalamic and colonic temperatures in calliope hummingbirds with high body temperatures is not different from that observed in other nonpasserines. The average body-to-brain temperature difference in nonpasserine birds ranges from 0.50 °C in pekin ducks (Schmidt and Simon 1979) to 1.29 °C in mallards (Kilgore et al. 1976). Also, in most species of birds studied the relationship between brain and body temperature has yielded a regression line with a slope not statistically different from that of the isothermal line (Kilgore et al. 1973, 1976, 1979; Bernstein et al. 1979b; Crowe and Withers 1979; Schmidt and Simon 1979; Bech and Midtgård 1981; Kleinhaus et al. 1985). In these birds, the brain is cooled just as much at high body temperatures as at lower body temperatures. Only in the kestrel (Bernstein et al. 1979a), bobwhite (Kilgore et al. 1981) and some fowls (Arad and Midtgård 1983) is there a statistically greater body-to-brain temperature difference at high body and air temperatures. Arad et al. (1984) have recently suggested that regressions of brain temperature on colonic temperature over a wide range of air temperatures may be misleading, since over narrower temperature ranges the slopes of these relationships may be significantly lower than unity, indicating

increased brain cooling at the higher body temperatures.

The relationship between brain and colonic temperature at low body temperatures ($<39.5^{\circ}\text{C}$) is represented by the following regression equation:

$$T_{br} = 0.39 T_{cl} + 24.10 \quad (s_{y \cdot x} / \bar{Y} = 0.02, r = 0.64).$$

The slope of this regression line is statistically different from unity ($P < 0.05$) and the relationship is linear ($F_{[1,10]} = 6.9, P = 0.025$). The mean body-to-brain temperature difference at $T_{cl} < 39.5^{\circ}\text{C}$ was -1.38°C ($SD = 1.0; n = 12$) and varied from -0.43 to -3.2°C (Fig. 2).

A consistent pattern of brain temperatures that exceed body temperature has not been reported previously in birds. Most bird species have a cooler brain at all body and air temperatures. However, Crowe and Withers (1979) did observe that brain temperature of individual guineafowl routinely exceed body temperatures by as much as 0.70°C . The absence of comparable results in the other species of birds studied to date, probably reflects the difference between body temperature regulation of these birds and calliope hummingbirds. Calliope hummingbirds are 'stubborn' homeotherms (Lasiewski 1963) maintaining low body temperatures over a range of air temperatures (Fig. 1), while the other birds studied are not capable of such regulated hypothermia.

The precise mechanism or mechanisms responsible for brain temperatures higher than those of the body are unclear. Brain temperature in birds is probably determined by metabolic heat production of brain tissue, cerebral blood flow, and the temperature of the blood supplying the brain, as it is in mammals (Baker 1982). Therefore, there seemingly are but a few likely explanations for how the brain could be at a higher temperature than colonic. Slight adjustments in both cerebral blood flow and endogenous heat production of brain cells may be all that is necessary to maintain brain temperature in face of a declining body temperature, but major changes in both parameters are probably not possible.

The most plausible explanation for the higher brain than body temperature in calliope hummingbirds with low colonic temperatures is that colonic temperature may not be equivalent to core temperature. If core temperature of hummingbirds is above colonic temperature, which is likely at low air temperatures, then the temperature of the arterial blood supplying the brain, and brain temperature would potentially exceed the cooler colonic

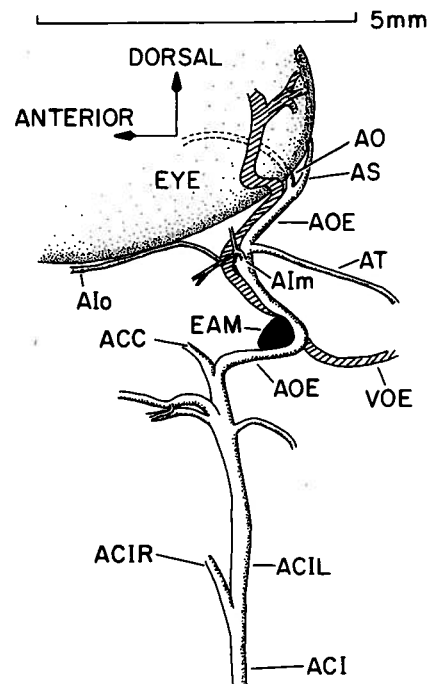


Fig. 3. Partially schematic left lateral view of the external ophthalmic artery and associated vessels ventral to the eye of the calliope hummingbird. Arteries are stippled, vein is cross-hatched: ACC A. carotis cerebialis; ACI A. carotis interna; ACIL A. carotis interna, left; ACIR A. carotis interna, right; Alo A. infraorbitalis; Alm A. intramandibularis; AO A. ophthalmotemporalis; AOE A. ophthalmica externa; AS A. supraorbitalis; AT A. temporalis; EAM external acoustic meatus; VOE V. ophthalmica externa

temperature. Lasiewski (1963) has shown that calliope hummingbirds exposed to low air temperatures have a reduced thermal conductance, one possible cause of which is peripheral cooling. Divergence of core and colonic temperatures might also explain why the body-to-brain temperature difference was greatest in calliope hummingbirds with the lowest colonic temperatures (Fig. 2).

Vascular anatomy

No distinct RMO was observed in the calliope hummingbird. This unicarotid species (Glenny 1955) has a single ventrally located internal carotid artery on the neck. This vessel branches in the region of the head to form a right and left internal carotid artery (Fig. 3). A branch of these, the external ophthalmic artery courses around the external acoustic meatus and parallels and touches the external ophthalmic vein. Posteroventral to the eye, the external ophthalmic artery sends off a small infraorbital artery that follows the orbit posteroventrally for a short distance. This and the small intramandibularis artery wrap around the external

ophthalmic vein at their root. A large branch of the external ophthalmic artery, the temporalis artery, passes caudodorsally to the temporal muscle. The external ophthalmic artery then continues to juxtapose the external ophthalmic vein for a short distance before it splits into the ophthalmotemporal artery that proceeds medial to the orbit, and the supraorbital artery that continues dorsally along the posterior surface of the eye. Posteromedial to the eyeball there is a plexus of small blood vessels lying between the branch of the supraorbital artery and the ophthalmotemporal artery (not shown on Fig. 3).

The anatomy of the blood vessels in the temporal region of the calliope hummingbird deviates significantly from that described in other birds in that a RMO is lacking. To our knowledge, this is the first authentication of the absence of this structure in any bird, although Saiff (1974) reported the absence of a rete mirabile in a non-injected specimen of the Peruvian diving-petrel.

RMO complexity and the magnitude of the body-to-brain temperature difference

Our data clearly indicate that the calliope hummingbird is able to cool its hypothalamus in the face of rising body temperature and yet lacks the vascular structure that is believed responsible for brain cooling in other birds. What is not clear is how brain cooling might be accomplished in the absence of a RMO. The most likely explanation is that even without a RMO, there is sufficient heat exchange between the arterial blood supply to the brain and cool venous blood, along both the anterior and/or posterior pathways of cerebral blood flow. Specifically, heat could be exchanged between the cerebral carotids and the sinus cavernosus (Richards 1970) and the external ophthalmic arteries and veins (Fig. 3). In this regard, the observed relationship between brain temperature and anatomy of the external ophthalmic blood vessels supports Midtgård's (1983) hypothesis that brain cooling is not solely a function of RMO structure, but rather heat exchange efficiency.

The results of the present experiments can not be explained by increased heat loss from the head of hummingbirds as appears to be the case in the naked-headed guineafowl (Crowe and Withers 1979). In birds with feathered heads, the brain appears to be well insulated (Warncke and Stork 1977). Furthermore, if there was a differentially high heat loss from the small heads of hummingbirds, relative to the body, one might realistically expect brain temperature to generally be below

that of the body, especially at low air temperatures where conductive heat loss would be enhanced.

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References

- Altman RB (1980) Avian anesthesia. *Comp Cont Educ* 38:38-42
- Arad Z, Midtgård U (1983) Brain cooling in dehydrated heat exposed fowls. *Physiologist* 26:A-30
- Arad Z, Midtgård U (1984) Differences in the structure of the *rete ophthalmicum* among three breeds of domestic fowls, *Gallus gallus f. domesticus* (Aves). *Zoomorphology* 104:184-187
- Arad Z, Toledo CS, Bernstein MH (1984) Development of brain temperature regulation in the hatchling mallard duck *Anas platyrhynchos*. *Physiol Zool* 57:493-499
- Baker MA (1982) Brain cooling in endotherms in heat and exercise. *Annu Rev Physiol* 44:85-96
- Baumel JJ (1979) *Systema cardiovasculare*. In: Baumel JJ, King AS, Lucas AM, Breazile JE, Evans HE (eds) *Nomina anatomica avium*. Academic Press, London, pp 343-407
- Bech C, Midtgård U (1981) Brain temperature and the *rete mirabile ophthalmicum* in the zebra finch (*Poephila guttata*). *J Comp Physiol* 145:89-93
- Bernstein MH, Curtis MB, Hudson DM (1979a) Independence of brain and body temperatures in flying American kestrels, *Falco sparverius*. *Am J Physiol* 237:R58-R62
- Bernstein MH, Sandoval I, Curtis MB, Hudson DM (1979b) Brain temperature in pigeons: effects of anterior respiratory bypass. *J Comp Physiol* 129:115-118
- Crowe TM, Withers PC (1979) Brain temperature regulation in helmeted guineafowl. *S Afr J Sci* 75:362-365
- Daniel WW (1978) *Applied nonparametric statistics*. Houghton Mifflin, Boston
- Eisenhart C (1968) Expression of the uncertainties of final results. *Science* 160:1201-1204
- Fedde MR (1978) Drugs used for avian anesthesia: a review. *Poultry Sci* 57:1376-1399
- Glenny FH (1955) Modifications of pattern in the aortic arch system of birds and their phylogenetic significance. *Proc US Natl Mus* 104:525-621
- Hudson DM, Clair PM, Bernstein MH (1985) Direct measurement of temperature gradients in the *rete ophthalmicum* of the double-crested cormorant (*Phalacrocorax auritus*). *Physiologist* 28:272
- Kilgore DL, Bernstein MH, Schmidt-Nielsen K (1973) Brain temperature in a large bird, the rhea. *Am J Physiol* 225:739-742
- Kilgore DL, Bernstein MH, Hudson DM (1976) Brain temperatures in birds. *J Comp Physiol* 110:209-215
- Kilgore DL, Boggs DF, Birchard GF (1979) Role of the *rete mirabile ophthalmicum* in maintaining the body-to-brain

- temperature difference in pigeons. *J Comp Physiol* 129:119–122
- Kilgore DL, Birchard GF, Boggs DF (1981) Brain temperatures in running quail. *J Appl Physiol* 50:1277–1281
- Kleinhaus S, Pinshow B, Bernstein MH, Degen AA (1985) Brain temperature in heat-stressed, water deprived desert phasianids: sand partridge (*Ammoperdix heyi*) and chukar (*Alectoris chukar sinaica*). *Physiol Zool* 58:105–116
- Kruger K, Prinzing R, Schuchmann KL (1982) Torpor and metabolism in hummingbirds. *Comp Biochem Physiol* 73A:679–689
- Lasiewski RC (1962) The capture and maintenance of hummingbirds for experimental purposes. *Avicult Mag* 68:59–64
- Lasiewski RC (1963) Oxygen consumption of torpid, resting, active, and flying hummingbirds. *Physiol Zool* 36:122–140
- Lasiewski RC (1964) Body temperatures, heart and breathing rate, and evaporative water loss in hummingbirds. *Physiol Zool* 37:212–223
- Midtgård U (1983) Scaling of the brain and the eye cooling system in birds: a morphometric analysis of the *rete ophthalmicum*. *J Exp Zool* 225:197–207
- Midtgård U, Arad Z, Skadhauge E (1983) The *rete ophthalmicum* and the relation of its size to the body-to-brain temperature difference in the fowl (*Gallus domesticus*). *J Comp Physiol* 153:241–246
- Pinshow B, Bernstein MH, Lopez GE, Kleinhaus S (1982) Regulation of brain temperature in pigeons: effects of corneal convection. *Am J Physiol* 242:R577–R581
- Richards SA (1970) Brain temperature and the cerebral circulation in the chicken. *Brain Res* 23:265–268
- Saiff EI (1974) The middle ear of the skull of birds. The Procellariiformes. *Zool J Linn Soc* 54:213–240
- Schmidt I, Simon E (1979) Temperature changes of the hypothalamus and body core in ducks feeding in cold water. *Pflügers Arch* 378:227–230
- Warncke VG, Stork HJ (1977) Biostatische und thermoregulatorische Funktion der sandwich-Strukturen in der Schädeldecke der Vögel. *Zool Anz* 199:251–257