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# METABOLIC COMPENSATION FOR GRADUAL COOLING IN DEVELOPING CHICK EMBRYOS

H. TAZAWA,* H. WAKAYAMA,* J. S. TURNER†‡ and C. V. PAGANELLI†

*Department of Electronic Engineering, Muroran Institute of Technology, Muroran 050, Japan; †Department of Physiology, Schools of Medicine and Dentistry, State University of New York at Buffalo, Buffalo, NY 14214, USA and ‡Perry Fitz Patrick Institute of African Ornithology, University of Cape Town, Rondebosch 7700, Republic of South Africa

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**Abstract**—1. Prior to around day 18 of incubation, chicken embryos are apparently poikilothermic. No compensatory increase of metabolic rate is evident when the embryo is gradually cooled.

2. At about day 18 of incubation, a weak metabolic response to egg cooling appears.

3. After external pipping, the metabolic response to gradual cooling is stronger. The embryo need not emerge from the egg for the compensation to occur.

4. We suggest that precocial hatchlings *in ovo* may exhibit incipient endothermic homeothermy. Full homeothermy may be prevented by the low gas conductance of the eggshell, effectively "throttling" the embryo's heat production capacity. This is a constraint altricial birds probably never experience.

## INTRODUCTION

Chicken embryos are poikilothermic during development; only after hatching does the ability to maintain a constant body temperature appear (Romijn and Lokhorst, 1955; Freeman, 1964; Wekstein and Zolman, 1967; Tazawa and Rahn, 1987). There is some ambiguity about how, or when, the transition from a poikilothermic embryo to a homeothermic and endothermic chick occurs. Some (Freeman, 1964) have reported that 19-day-old embryos respond to cooling of the egg with transient increase of oxygen consumptions presumably a compensatory increase of metabolic heat production. Others (Romijn and Lokhorst, 1955) have seen no signs of metabolic compensation, even in the full-term (20-day-old) embryo; in typical poikilotherm fashion, their $O_2$ consumption decreases with embryo temperature.

The design of these experiments may have made it very difficult to see a compensatory metabolic response. Let us illustrate why. If a chicken egg is exposed to air 10°C cooler than the egg's centre, the embryo will have to generate heat at a rate of about 800 mW to keep the egg temperature steady (Turner, 1986). Yet, a chicken embryo can generate at most about 130 mW (this paper). Consequently, rate of heat loss during cooling exceeds the embryo's maximum rate of heat production by at least 6-fold. If a feeble compensatory ability does exist in a late-term chicken embryo, it may be overwhelmed, or "swamped" by the very much larger losses of heat.

If the "swamping" problem can be minimized, a clearer picture may emerge of the embryo's metabolic responses to cooling of its egg. One way to minimize the "swamping" problem is to make the imbalance between heat loss and heat production as small as possible while the egg is cooling. This is the primary rationale for the experiments we describe in this paper.

## MATERIALS AND METHODS

Fertile chicken eggs were incubated at 38°C in a forced-draft incubator. One day prior to an experiment, a calibrated copper–constantan thermocouple, 0.8 mm in diameter, was implanted 1 cm inside the egg. The egg, along with its thermocouple, was returned to the incubator for 1 day until the experiment. The temperature of the egg could be measured with an accuracy of 0.1°C, using a Bailey BAT8 thermocouple thermometer.

Eggs were cooled in one of two ways. In "step-function cooling", the egg was moved abruptly from the 38°C incubator to a 28°C test chamber. The egg was then allowed to cool for about 5 hr. While the egg was cooling, its temperature was monitored continuously. We used step-function cooling to estimate the thermal conductance of eggs in our apparatus. "Gradual cooling" was the second method of cooling the egg. Here, the egg was moved from a 38°C incubator to a test chamber immersed in a water bath, also at 38°C. After the egg's temperature had equilibrated in the test chamber, the heaters and regulators of the water bath were turned off. We then allowed the water bath, along with the test chamber and egg, to cool gradually to room temperature. The average time constant of the water bath was about 5.6 hr, and we allowed the experiment to proceed for 7–8 hr. During gradual cooling, egg temperature lagged slightly behind the ambient temperature. While the egg cooled, its temperature and $O_2$ consumption were continually monitored.

The $O_2$ consumption was measured with a modified Scholander and Edwards respirometer (Scholander and Edwards, 1942), which was submerged in the water bath. It consisted of two Plexiglas chambers of equal volume, connected by a water-filled U-tube manometer. The living egg, along with a KOH solution, was placed in one chamber, and an infertile egg was placed in the other; the chamber with the infertile egg served as a compensating chamber. As the living egg consumed oxygen, the level of water in the manometer was displaced. The consumed oxygen was periodically (every 2 min during a 10–20 min interval) replaced by injecting $O_2$ from a syringe into the test chamber. The $O_2$ consumption, in ml STPD/day, was calculated by a regression equation between cumulative volume of $O_2$ injected and time.

## RESULTS

When eggs were exposed to a $-10$°C step change of ambient temperature, their cooling curves were, as expected, very close to exponential (Fig. 1). The time

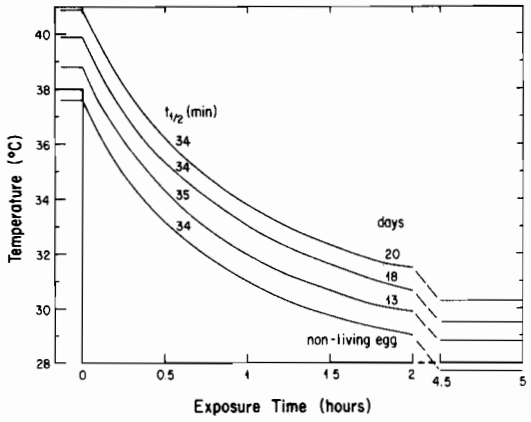


Fig. 1. Some representative cooling curves for eggs of different ages (in days) and non-living egg cooled by "step-function" method. The half time ($t_{1/2}$ in min) of responses is shown above the individual curves.

constant for cooling ($\tau = t_{\frac{1}{2}}/0.693$, where $t_{\frac{1}{2}}$ is half time of the response) did not change appreciably from day 12 to day 20 of incubation (Table 1). We may infer from this that the egg's thermal conductance also did not change appreciably during incubation. From the time constant along with the specific heat of the egg ($c$, 3313 J/kg per °C; Romanoff and Romanoff, 1949) and egg mass ($M$), the egg's thermal conductance ($G$ in mW/°C) may be calculated as $G = (1000/60) \cdot c \cdot M/\tau$. In our apparatus, egg conductances averaged about 72 mW/°C (Table 1).

When no embryo was present, the egg temperature was slightly below ambient temperature, owing to evaporation from the egg (Fig. 1). As the embryo developed, and the resting rate of metabolic heat production increased, the egg temperatures were elevated above ambient temperature (Table 1, Fig. 1). From this steady-state temperature difference ($\Delta T_i$) and from the egg's thermal conductance, the egg's heat production ($q$ in mW) may be estimated ($q = \Delta T_i \cdot G$). During the latter parts of the incubation period, the rate of heat production averaged about 130 mW. This value appeared to be very adequate when it was converted into the $O_2$ consumption (Table 1) which agreed very well with the previously reported value (Tazawa and Rahn, 1987).

When eggs were cooled gradually, the temperature difference between the egg and surroundings was always less than 3°C. Consequently, heat loss was, in general, less than double the rate of heat production. This contrasts with a −10°C step change, where heat loss is initially 6 times the rate of heat production (Table 1). Therefore, gradual cooling had the desired effect of reducing the imbalance between heat production and heat loss while the eggs were cooled.

When eggs were gradually cooled, their rates of metabolic heat production followed one of three patterns (Fig. 2). Prior to day 17 of incubation, metabolism declined virtually from the onset of cooling, roughly paralleling the decline of egg temperature (Fig. 2a). Between day 18 of incubation and the day the embryos pipped the eggs externally, there was a "plateau phase" in metabolism at the start of cooling (Fig. 2b). In this phase, metabolism remained essentially constant as egg temperature declined. The plateau phase ended when $O_2$ consumption began to decline, again roughly in parallel with the decrease of egg temperature (Fig. 2b). After the egg was externally pipped, there was a marked increase in metabolic rate at the start of cooling, which lasted until the egg had cooled to about 35–34°C, after which the metabolic rate declined in parallel with egg temperature (Fig. 2c). It should be noted that a chick which did not emerge from the shell at 22 days is included in Fig. 2c (marked with an asterisk).

## DISCUSSION

The transition from a poikilothermic embryo to a homeothermic hatchling is a two-part process. First, the embryo's nervous system must be sufficiently developed, so that the coordinated neural mechanisms necessary for thermoregulation, thermosensors and controllers, may work. Second, the embryo must develop the "effectors"; the thermogenic and thermolytic mechanisms, that enable the neural "controllers" to operate. Without both, endothermic homeothermy is not possible (Dawson and Evans, 1957; Hissa *et al.*, 1983; Marsh and Wickler, 1982).

It is well known that thermoregulatory abilities differ between altricial and precocial hatchlings (Ricklefs, 1974). Altricial hatchlings apparently do not have sufficient metabolic capacity to defend their body temperature, nor does their neural development appear to be sufficient for anything but behavioral thermoregulation. Altricial hatchlings are only capable of endothermic homeothermy days or weeks after hatching (Dunn, 1975; Hill and Beaver, 1982). Precocial hatchlings, in contrast, are often able to defend their body temperatures within hours of hatching (Hissa *et al.* 1983), or even immediately after hatching (Booth, 1984; Eppley, 1984; Koskimies and Lahti, 1964). It appears that the thermoregulatory mechanisms of a precocial bird are adequately developed at hatching, needing only to be "switched on" shortly after hatching.

Table 1. Summary of results from step-function cooling experiments

| Age (days) | 12 | 13 | 14 | 17 | 18 | 19 | E |
|---|---|---|---|---|---|---|---|
| *Measured quantities* | | | | | | | |
| No. | 2 | 2 | 2 | 6 | 6 | 7 | 9 |
| $\Delta T_i$ (°C) | 0.55 | 0.75 | 1.0 | 1.7 ± 0.4 | 1.9 ± 0.2 | 1.8 ± 0.4 | 2.2 ± 0.3 |
| $\tau$ (min) | 47.6 | 45.0 | 44.6 | 42.1 ± 4.0 | 41.2 ± 3.6 | 41.3 ± 1.3 | 41.2 ± 4.2 |
| *Calculated quantities* | | | | | | | |
| G (mW/°C) | 63.2 | 68.2 | 68.2 | 72.6 ± 6.2 | 73.9 ± 5.9 | 73.9 ± 2.8 | 74.1 ± 7.6 |
| q (mW) | 34.8 | 51.0 | 68.2 | 121.4 ± 29.9 | 136.7 ± 21.0 | 131.6 ± 30.4 | 163.4 ± 27.5 |
| $\dot{M}o_2$ (l/day) | 0.16 | 0.23 | 0.31 | 0.56 ± 0.14 | 0.63 ± 0.11 | 0.60 ± 0.15 | 0.75 ± 0.13 |

E, externally pipped eggs.

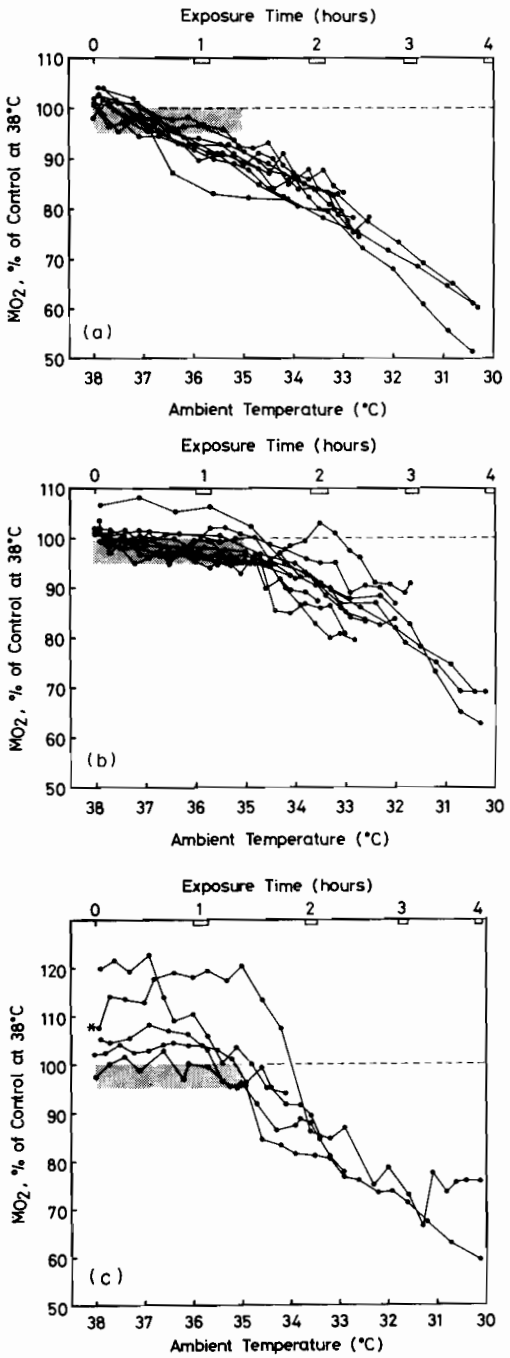


Fig. 2. Oxygen consumption for eggs cooled by the "gradual cooling" method. Dotted area indicates $O_2$ consumption maintained within 95% of control value despite a 3°C fall in ambient temperature. (a) Eggs younger than 17 days of incubation (2, 2, 1 and 4 eggs for days 12, 14, 16 and 17, respectively). (b) Eggs between 17 days of incubation and external pipping (1, 6, 2 and 2 eggs for days 17, 18, 19 and 20, respectively). (c) Eggs after external pipping but before hatching (3, 1 and 1 eggs for days 20, 21 and 22, respectively). The embryo indicated by the asterisk was still in the shell on day 22 of incubation.

Do the thermoregulatory differences between altricial and precocial birds extend to the embryo as well? An altricial embryo certainly is poikilothermic; the transition to homeothermy always takes place after the embryo hatches (Dunn, 1975; Hill and Beaver, 1982). Superficially, this appears to be the case for precocial embryos as well. We know of no case where a precocial egg has been shown capable of defending its own egg temperature. When exposed to cool conditions, precocial eggs invariably cool, as did the eggs in this study.

Nevertheless, we believe our data show that the thermoregulatory mechanisms of chicken embryos are actually "switched on" several days prior to hatching, even prior to external pipping. If an embryo has externally pipped the egg, but not yet hatched, its metabolic rate goes up in response to cooling, as if the embryo was metabolically defending its egg temperature (Fig. 2c). Prior to external pipping of the egg, but after the egg is at least 17 days old, the metabolic response to gradual cooling is a plateau in metabolic rate at the start of cooling; the metabolic rate is "uncoupled" from egg temperature, at least for the initial phase of cooling (Fig. 2b). This response differs markedly from the metabolic response to gradual cooling in younger eggs, where metabolic rate was strongly coupled to egg temperature (Fig. 2a).

If the thermoregulatory mechanisms of chicken embryos *in ovo* are operative, why do the embryos not thermoregulate? We believe the precocial embryo *in ovo* faces a constraint on thermoregulation that an altricial embryo never will. Our view of this constraint can best be illustrated using Fig. 3, which compares altricial and precocial embryos and hatchlings in their transition from poikilothermy to homeothermy.

Defining when a hatchling becomes homeothermic is somewhat arbitrary; for our discussion, we will adopt the convention that homeothermy occurs when the embryo's heat production rate reaches a certain level ("Minimum Heat Production for $\Delta T$"; Fig. 3). If an egg's thermal conductance does not change during incubation (Table 1), this level of heat production corresponds to the egg temperature being warmer than ambient temperature by some minimum amount, $\Delta T$. This is similar to criteria used by others (e.g. Dunn, 1975) to decide when altricial hatchlings may be considered endothermic homeotherms. It should be noted that the embryo's thermal conductance may change after hatching, whether because of evaporation from a wet, freshly-hatched bird, growth or insulation (e.g. Dawson and Bennett, 1981; Dawson *et al.*, 1976; Marsh, 1979). For simplicity, we ignore these post-hatching changes of conductance, for they make no difference to the important parts of our argument.

The eggshell gas conductance also may set a limit to the amount of heat an embryo can produce (Fig. 3). The diffusive conductance of eggshells to $O_2$ is essentially fixed as long as the eggshell is intact (Paganelli *et al.*, 1978; for an interesting exception, see Booth and Seymour, 1987). Therefore, the only way $O_2$ flux across the eggshell, and presumably the embryo's heat production, may be increased is to increase the diffusion gradient for $O_2$ (Visschedijk, 1980). Under "normal" conditions (i.e., air at 1 atm), this means that hypoxia is the price the embryo must pay to increase its heat production. There is presumably a limit to this, and this will impose a limit

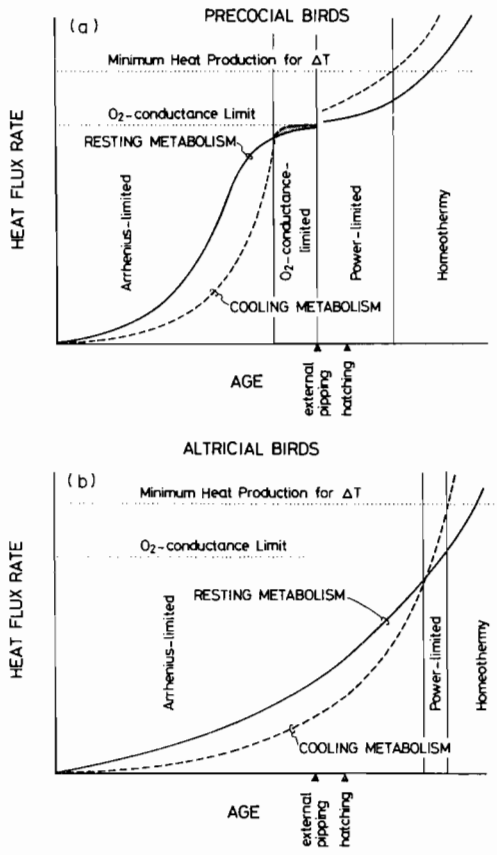


Fig. 3. Energetics of the transition from poikilothermy to endothermy. (a) The transition for a precocial bird. (b) The transition for an altricial bird. The figure is explained in the text.

on the amount of heat an embryo in an enclosed egg can produce ("$O_2$ Conductance Limit"; Fig. 3). Because eggshell gas conductances of precocial and altricial birds do not differ (Ar and Rahn, 1980), this limit will be the same for both altricial and precocial embryos.

We envisage that a precocial bird's transition from poikilothermy takes place in four stages. Each stage in the transition is characterized by its own limitations.

The first stage corresponds to most of the incubation period, when the embryo has neither sufficiently developed controllers nor effectors. The metabolic rate is low, but increases as the embryo grows ("Resting Metabolism"; Fig. 3; Hoyt and Rahn, 1980). If the surroundings cool, the egg cools with it, and the metabolic rate goes down by some proportion ("Cooling Metabolism"; Fig. 3). For chicken embryos, metabolism at 24°C is about 40% of the metabolism at 37°C (Tazawa and Rahn, 1987). This change is in rough conformity with the Arrhenius limitation of temperature on chemical kinetics. This stage we refer to as *Arrhenius-limited.*

Altricial and precocial hatchlings share an Arrhenius-limited period. They differ in that altricial hatchlings hatch while their metabolic responses to cooling are still Arrhenius-limited; in other words, they are still poikilothermic. We are suggesting that precocial hatchlings come out of the Arrhenius-limited stage while they are still in the egg.

For a precocial embryo, the second stage occurs from the latter part of the incubation period to external pipping. During this stage, the embryo's thermoregulatory control and effector mechanisms are sufficiently developed to be operative, but are "throttled" by the low conductance of the eggshell to $O_2$ diffusion. If the embryo's resting metabolic rate is still less than the eggshell's conductance limit, a slight increase in $O_2$ consumption might occur when the egg is cooled, but it will not exceed the $O_2$ conductance limit. If the embryo's resting metabolic rate is already at the conductance limit, a plateau, but not rise, in $O_2$ consumption will be observed. This may explain the different results of Freeman (1964), who saw an increase in metabolism in a cooling egg, and of Romijn and Lokhorst (1955), who did not. With respect to egg temperature, if the throttled heat production is still less than the minimum required for homeothermy, the egg still will cool. At some point, the embryo's temperature may decline sufficiently to "switch off" the thermoregulatory machinery. Subsequently, the metabolic rate will decline in parallel with egg temperature, as it does when the egg's energetics are Arrhenius-limited. This stage we refer to as "$O_2$ conductance-limited".

The existence of an $O_2$ conductance-limited stage is predicted on there being a well-developed thermoregulatory system that would operate well if it were not throttled by the eggshell. This appears to be the case with chicken embryos, as we believe our data and those of others (Dawes, 1981) show. However, altricial embryos will never pass through this stage, because the controllers and effectors do not develop sufficiently until after they have left the shell, and its presumed throttling effect. Therefore, the conductance-limited stage should be unique to precocial embryos.

After the embryo pips the eggshell, its metabolic rate is no longer throttled by the eggshell, because its gas exchange through the chorioallantois can be supplemented by breathing $O_2$-rich air through the lungs. If the embryo is still in the egg, and its egg is cooled, it could increase its metabolic rate to the maximum it is capable of, as we observed (Fig. 2c). If the embryo's maximum metabolic rate still produces less heat than that needed to offset heat loss, the egg will cool. In this stage, homeothermy evades the embryo because its capacity to generate heat is not sufficiently great. This stage might be called "power-limited".

A power-limited stage is one that almost all avian young will go through. Again, altricial young will inevitably pass through it after they hatch. Precocial young probably will pass through it after they pip the egg externally, and it will continue for some time after they hatch. Some very well-developed precocial young, such as ducks and waterfowl (Koskimies and Lahti, 1964), young brush turkeys (Booth, 1984), or young murrelets (Eppley, 1984) may be capable of full-blown thermoregulation immediately after hatching; these hatchlings apparently bypass the power-limited stage altogether.

The final stage begins when the young are capable of fully defending their body temperature and con-

tinues through adolescence into adulthood. This is full-blown homeothermy, and is common to both altricial and precocial birds.

If the eggshell does "throttle" the nascent thermoregulatory abilities of precocial young, this raises an interesting question. To put the question rhetorically, if a precocial embryo has developed the capability of independently regulating its own egg temperature, why should it not be homeothermic while it is in the egg? Why should the eggshell "throttle" it?

There are probably many answers to this question. At least one is that an eggshell "throttle" makes the parent's parcelling of energy into the egg much more predictable. A homeothermic egg would have to be provisioned with enough energy both to develop and to regulate egg temperature. To successfully bring an egg to hatching would require the energetic costs of both to be predictable. The energetic costs of development are fairly standard for all birds (Ar *et al.*, 1987; Vleck and Vleck, 1987), and so presumably are very predictable. But the likely metabolic costs for temperature regulation are probably much less predictable. When provisioning the egg, the parent would have to make a "weather forecast" of the likely temperature near the end of incubation. A mistake on the "forecast" might mean either the embryo has insufficient energy to develop, or energy may be put into one egg that could have been directed to more eggs.

It is well-established that the avian eggshell limits the diffusive loss of water vapour during incubation to roughly 15% of the egg's initial mass, regardless of size of the egg or life history of the species (Ar and Rahn, 1980). It seems the avian eggshell is "designed" to limit water vapour losses during incubation. Perhaps the avian eggshell is also "designed" to limit the expenditures of energy during the incubation of precocial embryos.

## REFERENCES


Ar A., Arieli B., Belinsky A. and Yom-Tov Y. (1987) Energy in avian eggs and hatchlings. Utilization and transfer. *J. exp. Zool.* **1,** 151–164.

Ar A. and Rahn H. (1980) Water in the avian egg. Overall budget of incubation. *Am. Zool.* **20,** 373–384.

Booth D. T. (1984) Thermoregulation in neonate malleefowl, *Leipoa ocellata*. *Physiol. Zool.* **57,** 251–260.

Booth D. T. and Seymour R. S. (1987) Effect of eggshell thinning on water vapor conductance of Malleefowl eggs. *Condor* **89,** 453–459.

Dawes C. M. (1981) The effects of cooling the egg on the respiratory movements of the hatchling fowl, *Gallus gallus domesticus*, with a note on vocalization. *Comp. Biochem. Physiol.* **68A,** 399–404.

Dawson W. R. and Bennett A. F. (1981) Field and laboratory studies of the thermal relations of hatchling western gulls. *Physiol. Zool.* **54,** 155–164.

Dawson W. R., Bennett A. F. and Hudson J. W. (1976) Metabolism and thermoregulation in hatchling ring-billed gulls. *Condor* **78,** 49–60.

Dawson W. R. and Evans F. C. (1957) Relation of growth and development to temperature regulation in nestling field and chipping sparrows. *Physiol. Zool.* **30,** 315–327.

Dunn E. H. (1975) The timing of endothermy in the development of altricial birds. *Condor* **77,** 288–293.

Eppley Z. A. (1984) Development of thermoregulatory abilities in Xantu's murrelet chicks, Synthliborampus hypoleucus. *Physiol. Zool.* **57,** 307–317.

Freeman B. M. (1964) The emergence of the homeothermic-metabolic response in the fowl (*Gallus domesticus*). *Comp. Biochem. Physiol.* **A13,** 413–422.

Hill R. W. and Beaver D. L. (1982) Inertial thermostability and thermoregulation in broods of redwing blackbirds. *Physiol. Zool.* **55,** 250–266.

Hissa R., Saarela S., Rintamaki H., Linden H. and Hohtola E. (1983) Energetics and development of temperature regulation in capercaillie, *Tetrao uroqallus*. *Physiol. Zool.* **56,** 142–151.

Hoyt D. F. and Rahn H. (1980) Respiration of avian embryos. A comparative analysis. *Respir. Physiol.* **39,** 255–264.

Koskimies J. and Lahti L. (1964) Cold-hardiness of the newly hatched young in relation to ecology and distribution. *Auk* **81,** 281–307.

Marsh R. L. (1979) Development of endothermy in nestling bank swallows (*Riparia riparia*). *Physiol. Zool.* **52,** 340–353.

Marsh R. L. and Wickler S. J. (1982) The role of development in the transition to endothermy in nestling bank swallows, *Riparia riparia*. *J. Comp. Physiol.* **149,** 99–105.

Paganelli C. V., Ackerman R. A. and Rahn H. (1978) The avian egg. *In vivo* conductances to oxygen, carbon dioxide and water vapor in late development. In *Respiratory Function in Birds, Adult and Embryonic* (Edited by Piiper J.), pp. 212–218. Springer, Heidelberg.

Ricklefs R. E. (1974) Energetics of reproduction in birds. In *Avian Energetics* (Edited by Paynter R. A.), Vol. 15, pp. 152–192. Publications of the Nuttal Ornithological Club.

Romanoff A. L. and Romanoff A. T. (1949) *The Avian Egg*. John Wiley, New York.

Romijn C. and Lokhorst W. (1955) Chemical heat regulation in the chick embryo. *Poult. Sci.* **34,** 649–654.

Scholander P. F. and Edwards G. A. (1942) Volumetric microrespirometer for aquatic organisms. *Rev. Sci. Inst.* **13,** 292–295.

Tazawa H. and Rahn H. (1987) Temperature and metabolism of chick embryos and hatchlings after prolonged cooling. *J. exp. Zool.* **1,** 105–110.

Turner J. S. (1986) Cooling rate and size of birds' eggs—A natural isomorphic body. *J. therm. Biol.* **10,** 101–104.

Visschedijk A. H. J. (1980) Effects of barometric pressure and abnormal gas mixtures on gaseous exchange by the avian embryo. *Am. Zool.* **20,** 469–476.

Vleck C. M. and Vleck D. (1987) Metabolism and energetics of avian embryos. *J. exp. Zool.* **1,** 111–125.

Wekstein D. R. and Zolman J. F. (1967) Homeothermic development of the young chick. *Proc. Soc. exp. Biol. Med.* **125,** 294–297.