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Heat exchange and metabolic response to gradual cooling in developing chick embryos

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Abstract

- (1) The large heat conductance of incubating eggs indicates that heat loss upon step-function exposure to even mild cold (1.2°C difference) exceeds metabolic heat production of developing embryos.
- (2) Determination of O₂ consumption during gradual cooling of the egg suggests that a weak metabolic response emerges at about day 18 of incubation.
- (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) Thiourea which antagonizes the metabolic effects of thyroid hormones impaires this metabolic response.
- (5) 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.

1. Introduction

There is no doubt that chickens are dominantly poikilothermic during embryonic stages and develop a marked capacity to maintain body temperature after the time corresponding to hatching (Romijn and Lokhorst, 1955; Freeman, 1964, 1967; Wekstein and Zolman, 1967; Tazawa and Rahn, 1987). When it comes to the emergence of metabolic compensation to cooling during embryonic development, experimental results are ambiguous. It has been reported that when embryos reach 19 days of incubation, they respond to cold with a transient increase in O₂ consumption (Freeman, 1964), while in other reports (Romijn and Lokhorst, 1955) this apparent metabolic compensation does not appear, even in the full term embryo. Rather, O₂ consumption decreases upon cold exposure, reacting to cooling as younger embryos do. To examine the metabolic response of developing embryos to cold, a magnitude of cold relative to a metabolic heat production of embryos may be substantial. The present study was therefore designed, at first, to estimate the magnitude of heat conductance of eggs which allows the heat loss upon cold exposure to exceed the metabolic heat production. Secondly, based on the result of the first experiment, the gradual cooling procedure was designed to make the difference between heat loss upon cooling and heat

production very small and examine whether or not a metabolic compensation to cooling emerges before hatching. The result suggested that the metabolic response emerges before hatching. Furthermore, it has been reported that the thyroid hormone is involved in the thermoregulation of newly hatched chicks, which further prompted us to examine if the thyroid hormone is related to this metabolic response of embryos. The third experiment was therefore designed to determine the metabolic response of embryos which were given thiourea antagonizing the metabolic effects of thyroid hormones. Lastly, we envisioned the transition from poikilothermy to homeothermy in pre-social and altricial birds.

2. Methods and materials

Determination of egg heat conductance Fertile chicken eggs were incubated at 38°C in a forced draft incubator. One day prior to measurement of egg temperature, a calibrated copper-constantan thermocouple, 0.8mm in diameter, was implanted 1cm inside the egg. The egg, along with its thermocouple, was returned to the incubator. The temperature was measured to 0.1°C (model BAT8, Instrument Lab., U.S.A.). The following day, the equilibrium temperature of the egg in the 38°C incubator was recorded and then the egg was moved to a 28°C chamber. The egg temperature was continuously recorded during the next 5 hours. The cooling curve to this step-function change in ambient temperature was used for calculating the egg heat conductance. When the cooling curve is approximated by Newton's law of cooling, the coefficient of cooling rate (k) can be calculated from egg temperature at time t (T(t)) as follows,

$$k = -(1/t) \ln\{T(t) - (T_{ex} + \Delta T_i)\} / \{T_o - (T_{ex} + \Delta T_i)\} \quad (1)$$

where T_o is equilibrium temperature of the egg in 38°C ambience, ΔT_i is temperature difference between egg and ambience and T_{ex} is exposing temperature. The reciprocal of k is a thermal time constant (τ) which is given by a product of heat resistance (R) and heat capacitance (C) of the egg ($\tau = RC$). Suppose the heat capacitance ($C = \rho \cdot c \cdot V$, where ρ = egg density (1.035g/cm³), c = specific heat (0.8 cal/(g·°C)) and V = egg volume (60cm³)) is 50 cal/°C, the heat conductance ($G = 1/R$ in cal/(min·°C)) is approximated from the cooling rate coefficient multiplied by 50. The value was converted to SI units (mW/°C) dividing by 14.32×10^{-3} .

Determination of oxygen consumption during gradual cooling The oxygen consumption (\dot{M}_{O_2}) was measured with a modified Scholander and Edwards respirometer (Scholander and Edwards, 1942) which was submerged in the thermostatted water bath (45 × 45 × 30cm³). Gradual cooling of the egg was accomplished by turning off the temperature regulator of this water bath. The average

Heat exchange and metabolic response to gradual cooling in developing chick embryos

cooling rate coefficient of the water bath plus eggs was $0.18^{\circ}\text{C}/(\text{hr}\cdot^{\circ}\text{C})$ and it took 7–8 hours to cool to room temperature. This procedure made the difference between the heat loss estimated from the egg heat conductance and the heat production small.

The egg respirometer consisted of two plexiglass chambers of equal size, connected by a water-filled U-shaped manometer. The experimental egg, along with a KOH solution, was placed in one chamber and a non-living egg was placed in the other (compensating chamber). When the water bath was allowed to cool, the temperature change in the egg of the experimental chamber was compensated by that of the non-living egg, which had similar cooling time constant. Furthermore, the thermocouples installed in both egg chambers recorded temperatures continuously and showed no significant difference during the cooling phase. As embryos consumed O_2 , the level of water in the manometer was displaced, which was periodically corrected by injecting O_2 during 10 or 20 min. The time and volume of O_2 injected were recorded and a regression equation was calculated for the variables after the volume was reduced to STPD. The $\dot{M}\text{O}_2$ was given by a slope of regression equation and the value was expressed in l/day.

Administration of thiourea Treatment of eggs with anti-thyroid hormone (thiourea) followed Wittmann *et al.* (1984). A 50 mg-thiourea was solved in 5 ml-saline. On day 17 of incubation, a small hole was made in the eggshell and 0.25 ml-thiourea solved saline (32.8 micromol thiourea) was injected into the allantoic fluid. The hole was covered with glue after administration.

3. Results

Heat conductance of the egg Examples of cooling curves in the developing embryo and non-living

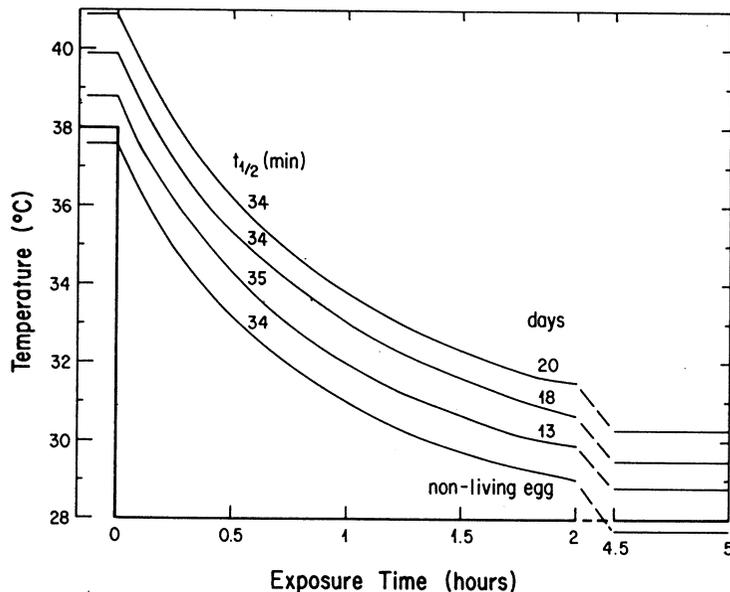


Fig. 1. Some representative cooling curves for eggs of different ages (in days) and non-living egg cooled by "step-function" method. The half time ($t_{1/2}$ in min) of responses is shown above the individual curves.

egg which were exposed to step-function decrease in ambient temperature are shown in Fig. 1. Due to the metabolism, the egg equilibrium temperature in 38°C (referred to as T_e) was higher than the ambient, a difference of which increased with embryonic age (referred to as ΔT_i). The non-living (dead) egg had, on the other hand, equilibrium temperature below the environment because of evaporative heat loss and lack of metabolic activity. Upon exposure to low environmental temperature (referred to as T_{ex}), the egg cooled exponentially and 4–5 hours later egg temperature reached a plateau (referred to as the quasi-equilibrium temperature). The cooling rate coefficient was calculated from eq. (1) and the time constant (reciprocal of cooling rate coefficient) is shown in Table 1 along with ΔT_i . The heat conductance (G) was approximated by dividing the heat capacity by time constant (Table 1).

Metabolic response to gradual cooling Eighteen eggs, ranging in ages from 12 days to externally pipping, were subjected to measurement of $\dot{M}O_2$ during gradual cooling. The measurement of $\dot{M}O_2$ was repeated for some eggs on different incubation days. The metabolic responses to gradual cooling which were determined for embryos before external pipping were sorted out into two groups based upon the following criterion in order to discriminate development of responses. The criterion used for discrimination was the oxygen consumption which was decreased below or main-

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

| Age (days) | 12 | 13 | 14 | 17 | 18 | 19 | E |
|-------------------------------------|------|------|------|----------------|----------------|----------------|----------------|
| <u>Measured quantities</u> | | | | | | | |
| No. | 2 | 2 | 2 | 6 | 6 | 7 | 9 |
| ΔT_i (°C) | 0.55 | 0.75 | 1.0 | 1.7 ±0.4 | 1.9 ±0.2 | 1.8 ±0.4 | 2.2 ±0.3 |
| τ (min ⁻¹) | 47.6 | 45.0 | 44.6 | 42.1 ±4.0 | 41.2 ±3.6 | 41.3 ±1.3 | 41.2 ±4.2 |
| <u>Calculated quantities</u> | | | | | | | |
| G (mW·°C ⁻¹) | 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 ⁻¹) | 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

tained within 95% of the control value during 3°C-fall in ambient temperature. Fig. 2a shows $\dot{M}O_2$ responses decreased below 95% of the control prior to 3°C-fall in ambient temperature. Embryos sorted out into this group are two 12-day, two 14-day, one 16-day and four 17-day old ones. Embryos whose $\dot{M}O_2$ responses which were maintained within 95% of the control after the ambient temperature fell by 3°C are presented in Fig. 2b. They are one 17-day, six 18-day, two 19-day and two 20-day embryos. The $\dot{M}O_2$ responses of five externally pipping embryos are shown in Fig. 2c.

As Wittmann *et al.* (1983) pointed out, embryos treated with thiourea were prevented from hatching and stayed within the shell up to 23 days of incubation. Fig. 2d represented the $\dot{M}O_2$ responses to gradual cooling determined for thiourea-treated embryos

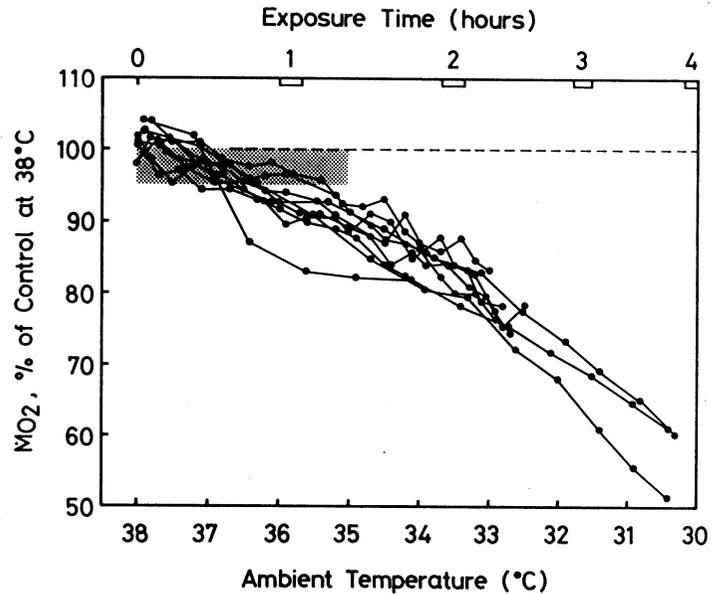


Fig. 2.a. Oxygen consumption for eggs cooled by the "gradual cooling" method. Dotted area indicates O₂ consumption maintained within 95% of control value despite a 3°C fall in ambient temperature. (a) Eggs younger than 17 days of incubation (2, 2, 1 and 4 eggs for days 12, 14, 16 and 17, respectively).

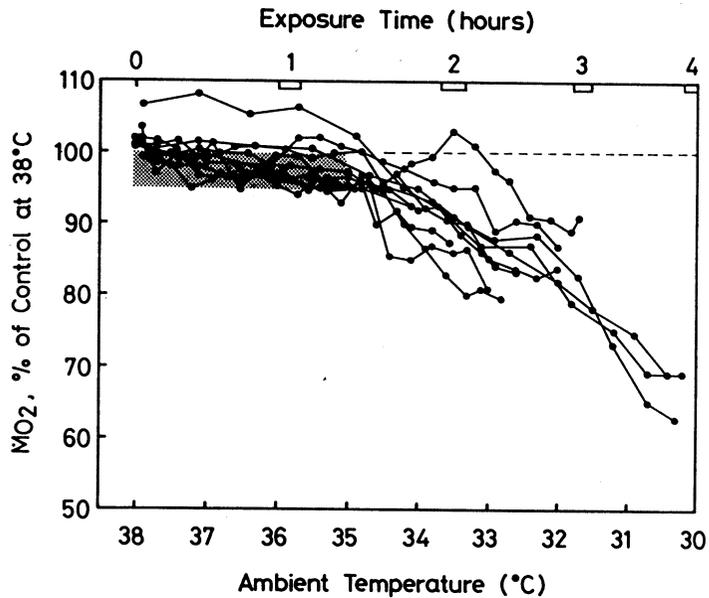


Fig. 2.b. (b) Eggs between 17 days of incubation and external pipping (1, 6, 2 and 2 eggs for 17, 18 19 and 20, respectively).

(two 21-day, one 22-day and one 23-day old).

4. Discussion

Heat conductance of the egg and comparison between heat production and heat loss upon cold exposure

During embryonic stages, eggs cooled with almost identical rates, irrespective of developmental stages and even external pipping (Fig. 1 and Table 1). The heat conductance of the egg approximated from the cooling rate coefficient results in approximately 70 mW/°C. These values indicate that when eggs are exposed to 28°C environment, at the beginning of exposure they lose the heat of about 700 mW.

On the other hand, because the equilibrium temperature of the egg at 38°C exceeds by ΔT_i above environment due to metabolic heat production against constant heat loss, the metabolic heat is estimated by

$$\dot{q} = \Delta T_i \cdot c \cdot M / \tau \quad (2)$$

where c and M are specific heat and egg mass (in g). From eq. (2), it is calculated that the embryo shown in Fig. 1 produces the metabolic heat at rates of about 50, 112 and 189 mW at days 13, 18 and 20 of in-

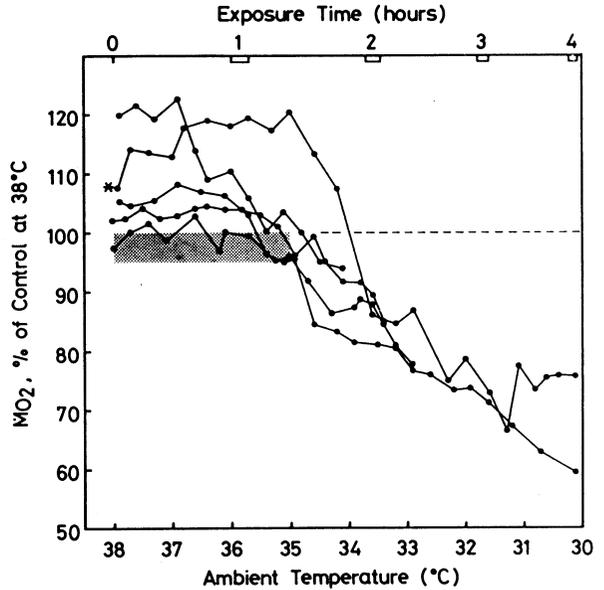


Fig. 2.c. (c) Eggs after external pipping but before hatching (3, 1 and 1 eggs for days 20, 21 and 22, respectively). The embryo indicated by the asterisk was still in the shell on day 22 of incubation.

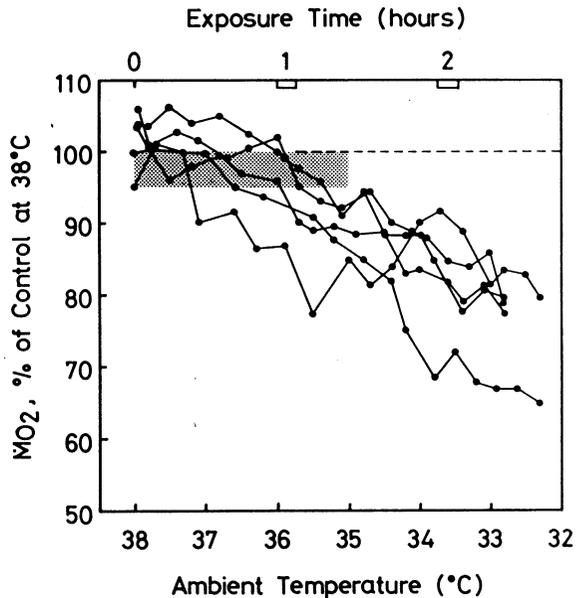


Fig. 2.d. (d) Eggs treated with thiourea (2, 2 and 1 eggs for days 21, 22 and 23, respectively).

incubation, respectively. The metabolic heat production divided by oxycalorific equivalent (4.5 cal/min, Brafield and Solomon, 1972) gives $\dot{M}O_2$ at equilibrium state; 0.22, 0.52 and 0.83 l/day at days 13, 18 and 20, respectively, for the embryo shown in Fig. 1. The O_2 consumption calculated as above for embryos subjected to step-function decrease in ambient temperature are shown in Table 1, which is approximately identical with previously observed values (Romanoff, 1967; Visschedijk, 1968; Bissonnette and Metcalfe, 1978; Ackerman and Rahn, 1981; Tazawa and Rahn, 1987).

Comparison between heat conductance and heat production of developing embryos suggests that the heat loss caused by ambient temperature drop of 1-1.5°C is almost comparable to the heat production of late embryos during early period of exposure and exceed that of younger embryos. Exposure to ambience whose temperature is a few degrees centigrade lower than incubator temperature causes the heat loss to exceed the heat production. Therefore, a step-function decrease in ambient temperature more than a few degrees centigrade may not be adequate to determine an emergence of metabolic response of developing embryos to cooling, because the large heat losses may overwhelm it.

Responses to gradual cooling in ambient temperature Calculation of heat loss conductance indicated that the heat lost at the beginning of exposure to 0.5-1.5°C step-function decrease in ambience is almost equal to metabolic heat produced by embryos developing during last half of incubation period. This suggests the gradual cooling of the surrounding may make the difference between heat loss and heat production very small throughout the temperature transient. This "drawing out" of the cooling curve may make it easier to see whatever small metabolic response might occur. This appears to occur in late term embryos. The $\dot{M}O_2$ response to gradual temperature drop is different in eggs of different developmental stages (Figs. 2a-2d). The $\dot{M}O_2$ which decreased gradually with decrease in ambient temperature was observed in younger embryos including four 17-day old ones. As embryos grew to near the end of prenatal period (about 18-19 days and onwards), a plateau of $\dot{M}O_2$ (defined as $\dot{M}O_2$ kept within 95% of control) is evident during at least the first one hour of exposure, while ambient temperature decreased by 3°C. Because eggs were cooled as second-order system, the plateau of metabolic rate which appeared in the first stages of cooling might be claimed not to an evidence of metabolic compensation, but a result of the second-order response of heat exchange. This is not the case, because there is no such plateau in $\dot{M}O_2$ in younger embryos. In addition, the plateau of $\dot{M}O_2$ which appeared in near term embryos does not owe to their metabolism which is enhanced with age towards the end of incubation, for this enhanced metabolism becomes almost identical value from 16-17 days up to external pipping.

The condition that changes in the environmental temperature had no effect upon metabolic rate

was termed the neutral condition (ref. Freeman, 1964). The neutral condition has been reported for the paranatal embryos which were exposed to step-function decrease in ambient temperature by 2.7°C (Freeman, 1964). In the present experiment whose cooling procedure was not made by step-function, little change in \dot{M}_{O_2} was observed already the late prenatal embryos which were exposed to temperature lowering by 3°C. Romijn and Lokhorst (1955) failed to see any metabolic reaction in full term embryos when they were exposed to step-function cooling over 3°C. However, they were suggested to have certain indications of metabolic compensation (homeothermia), a rise of the respiratory quotient during cooling over 10°C.

Although the metabolism tends to be maintained constant against gradual cooling of ambience in late prenatal embryos, this metabolic response is not large enough to sustain egg temperature. Fig. 3 shows exemplification on egg temperature changes measured simultaneously with \dot{M}_{O_2} response of day 14 and 18 embryos exposed to gradual cooling. Response of 14 and 18 days-old embryos are representative for young and near term embryos. The ambient temperature required about 6 hours to decrease from 38°C to 28°C. The broken line is a temperature isopleth indicating that egg temperature is equal to ambient temperature. When a fresh egg was measured for temperature 1 cm inside after egg contents were removed, leaving only the eggshell and

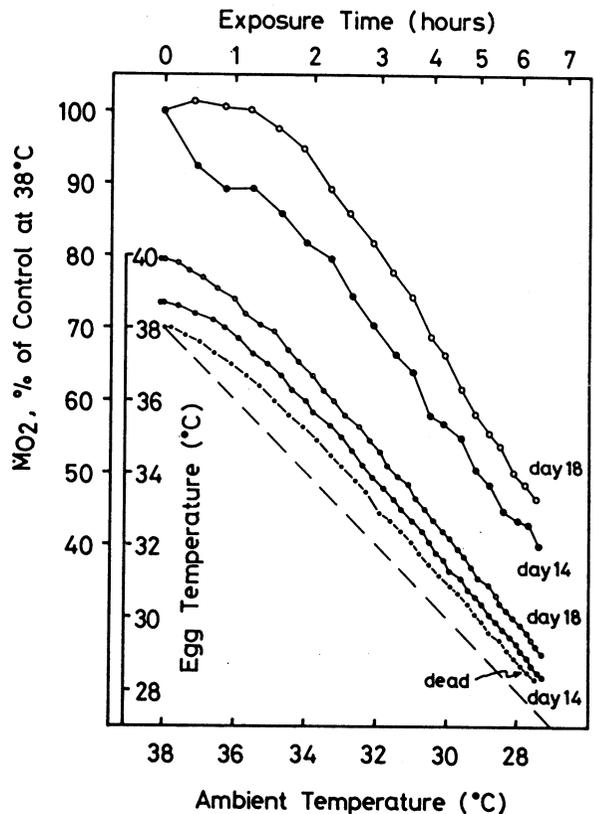


Fig. 3. Oxygen consumption (\dot{M}_{O_2}) and egg temperature changes, determined at days 14 and 18 of incubation, upon gradual decrease in environmental temperature. The \dot{M}_{O_2} is expressed as a ratio (in %) to control value at 38°C, and each plot indicates the average determined for 20 min. A broken diagonal line shows temperature isopleth indicating that egg temperature is equal to ambient temperature. Egg temperature was plotted every 10 min against ambient temperature.

its membrane, the temperature change followed this line. Because egg chamber was saturated with water vapor to 100% and thus little evaporation occurred, the dead egg temperature was the same as environment during equilibrium at control temperature (38°C). With gradual cooling, it deviated from a temperature isopleth because of large heat capacity of the egg compared with air. In living eggs, the temperature response curve started from further high temperature because of increasing heat production, but gradually decreased with time. After prolonged exposure, living eggs still maintained a temperature higher than the dead and the surroundings because of metabolism, although it was much reduced.

The metabolic responses to gradual cooling are augmented in externally pipping embryos, but the egg temperature is not still sustained even during early period of cooling exposure.

The metabolic response to cooling which is large enough to sustain egg temperature appears after the time corresponding to hatching. Fig. 4 shows the $\dot{M}O_2$ and egg temperature response to gradual cooling on day 22 of incubation. This egg was subjected to the same measurement during internal pipping on day 20. The chick was supposed to hatch one day prior to this measurement, but was still in the shell until 2.5 hours later. The $\dot{M}O_2$ increased upon exposure to ambient gradual cooling up to about 10% above the control. Initially, egg temperature was increased slightly during early period of exposure. However, the increase in metabolism was not yet large enough to maintain further the temperature which was decreasing in the meantime. The precipitous decrease in $\dot{M}O_2$ occurred when the shell was separated and again after the chick emerged from the shell, which implies that the augmented latent heat loss of vaporization overburdens the chick's metabolic capacity for heat production.

The effect of thiourea on metabolic response While oxygen consumption of embryos at 38°C averaged out to 0.54 (N=5), 0.56 (N=6), 0.64 (N=4) and 0.92 (N=5) l/day for 17-day, 18-day, internally pipping and externally pipping embryos, respectively, the average $\dot{M}O_2$ of thiourea treated embryos before cooling exposure was 0.74 (N=5) l/day at day 21-23. The $\dot{M}O_2$ of treated embryos become large compared with that of late prenatal embryos, while it was prevented from increasing compared with externally pipping embryos. Nevertheless, the response to cooling decreased with time as younger embryos did (Fig. 2d), implying that thiourea prevents from metabolic compensation for cooling which begins to emerge during late periods of prenatal development. The metabolic compensation is thus related to thyroid hormone and non-shivering.

Envisioning for the transition from poikilothermy to homeothermy 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 "effecters"; 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,

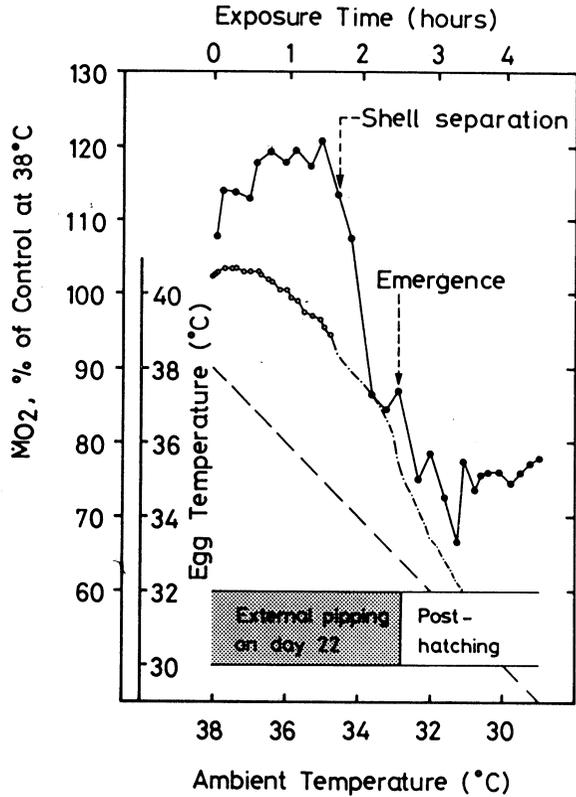


Fig. 4. Oxygen consumption (\dot{M}_{O_2}) and egg temperature changes of the chick which failed to emerge from the shell until it escaped on day 22 of incubation. The \dot{M}_{O_2} was plotted every 10 min. Open circles are plotted every 5 min for egg temperature until chicks separate the shell. After shell separation, temperature drops precipitously, reflecting that the environmental air penetrates into the egg. The \dot{M}_{O_2} is increased upon cooling which elevates egg temperature slightly at the beginning. Then, egg temperature decreases despite that \dot{M}_{O_2} is still kept above the control. The \dot{M}_{O_2} decreases in parallel with shell separation and again emergence from the shell, and levels off at about 70% of the pre-cooling (control) values.

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.

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 actually are "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 not the embryos 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 Figs. 5a and 5b, which

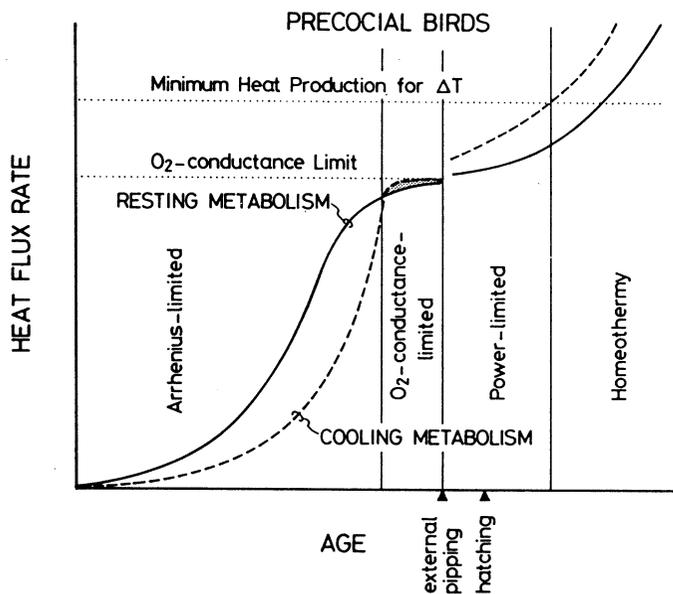


Fig. 5.a. Energetics of the transition from poikilothermy to endothermy. (a) The transition for a precocial birds.

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 ΔT "; Fig. 5). If an egg's thermal conductance does not change during incubation (Table 1), this level of heat production corresponds to the egg tempera-

ture being warmer than ambient temperature by some minimum amount, ΔT . 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. 5). 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" condition (*i.e.*, air at 1 atmosphere), 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 on the amount of heat an embryo in an enclosed eggs can produce (" O_2 Conductance Limit"; Fig. 5). Because eggshell gas conductances of precocial and altricial birds do not differ (Ar and Rahn, 1980),

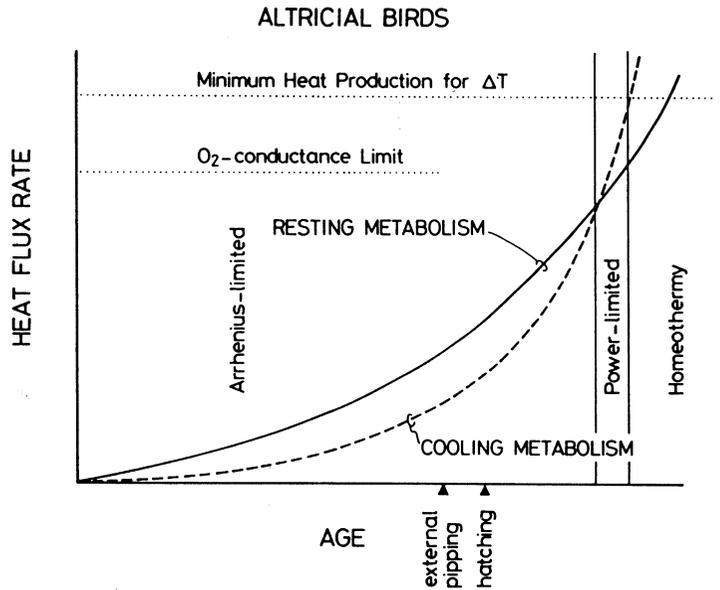


Fig. 5.b. (b) The transition for an altricial birds. The figure is explained in the text.

this limit will be the same for both altricial and precocial embryos.

We envision 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 effecters. The metabolic rate is low, but increases as the embryo grows ("RESTING METABOLISM"; Fig. 5; 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. 5). 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 O₂ diffusion. If the embryo's resting metabolic rate is still less than the eggshell's conductance limit, a slight increase in O₂ consumption might occur when the egg is cooled, but it will not exceed the O₂ conductance limit. If the embryo's resting metabolic rate is already at the conductance limit, a plateau, but no rise, in O₂ 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 *O₂ conductance-limited*.

The existence of an O₂ conductance-limited stage is predicted on there being a well-developed thermoregulatory system that would operate perfectly 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 never will pass through this stage, because the controllers and effecters do not develop sufficiently until after they have left the shell, and its pre-

sumed 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 O₂-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 continues 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 vapor 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 vapor losses during incubation. Perhaps the avian eggshell is also "designed" to limit the expenditures of energy during the incubation of precocial embryos.

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