Daily Energy Budgets of Avian Embryos: The Paradox of the Plateau Phase in Egg Metabolic Rate

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ABSTRACT

The metabolic rate of precocial bird eggs reaches a plateau when about 80% of the incubation period has passed. This is unexpected, as in many species the embryo continues to grow and maintenance costs must therefore increase. To investigate this paradox, daily energy budgets were constructed for embryos of four galliform species according to two models that used empirical data on egg metabolic rate and embryo growth. In the first model, embryonic synthesis costs were estimated, with an assumed synthesis efficiency, before calculating the maintenance costs. In the second model, embryonic maintenance was calculated first, and no assumptions were made on the synthesis efficiency. The calculations show that assumptions of the synthesis efficiency had a major impact on the energy budget calculations, because embryonic growth rate was high. During the plateau phase, a galliform embryo allocated energy in favor of its maintenance costs in three ways: by decreasing growth rate, by increasing synthesis efficiency, and by depressing the formation of glycogen. Our study suggests that a reduction in growth rate plays a minor role. An increase of synthesis efficiency is more likely to explain the plateau in energy expenditure, since small increases in synthesis efficiency can lead to great savings on synthesis costs.

Introduction

In avian eggs, the extraembryonic membranes (amnion, yolk sac membrane, and chorioallantois) need to be fully developed before embryonic mass can rapidly increase (Romanoff 1967). In Galliformes, this occurs when 60% of the incubation period has passed (see, e.g., Romijn and Lokhorst 1951; Romanoff 1967; Ancel 1989; Spiers and Baummer 1990), and the absolute growth rate is expected to be very high during the latter half of the incubation period. Likewise, the energetic costs of growth will be high. In Galliformes and other precocial birds, the metabolic rate of eggs reaches a plateau (or even decreases) after about 80% of the incubation period (C. M. Vleck et al. 1980). D. Vleck et al. (1980) pointed out that if embryonic growth continues during the plateau phase in metabolic rate, there is an energetic paradox: increasing embryo mass would imply increasing maintenance costs with a continuation of growth costs, and thus an egg metabolic rate that also increases. In view of the plateau, D. Vleck et al. (1980) concluded that embryonic growth rate must decline rapidly during the plateau phase. However, in most Galliformes, growth rate decreases only slightly or not at all during the plateau phase (see, e.g., Romijn and Lokhorst 1951; Romanoff 1967; Ancel 1989; Spiers and Baummer 1990; this study). This begs the question as to how the energy budget of a galliform egg is balanced during the plateau phase.

It is difficult to estimate the variation of the growth and maintenance components of embryonic metabolism during development. Models on the relationship between egg metabolism and embryonic mass and growth were previously published by Hoyt (1987) and Zonneveld and Kooijman (1993). Hoyt (1987) used an extended allometric model to describe the development of egg metabolic rate, including dry embryo mass and growth. The dynamic energy budget model of Zonneveld and Kooijman (1993) is more complicated and describes growth and storage use (yolk) simultaneously with the development of egg metabolic rate. Both models, however, use the whole data set, thereby smoothing day-to-day variances. These models also assume that mass-specific costs of growth are constant throughout incubation and neglect the metabolism of the extraembryonic membranes. The construction of empirically based daily energy budgets may overcome these problems and give insight into the day-to-day variation of embryonic growth and maintenance costs.

The daily energy expenditure of an egg can be divided into five cost factors: (1) maintenance of the embryo, that is, the metabolic costs to maintain the embryonic tissue in a steady state; (2) synthesis costs, that is, the costs of depositing new embryonic tissue and thus not the energy content of the deposited tissues (which is here factor 5); (3) muscular activity (particularly during the hatching process); (4) growth and metabo-
Metabolic Rate

Metabolic rates of the eggs were measured daily during development through hatching. In turkeys, metabolic rate was measured on pairs of eggs initially and, from day 11 onward, on individual eggs or hatchlings (174 eggs in total). In guinea fowl, metabolic rate was determined in pairs of eggs initially and, after day 19, in individual eggs or hatchlings (186 eggs in total). In quail, metabolic rate was measured on groups of three or four eggs initially, on pairs on days 12 and 13, and on individual eggs or hatchlings thereafter (142 eggs in total). When metabolic rate was measured on a group of eggs, fresh mass and water vapor conductance of the eggs were within the 5% range around the mean values of the group. Eggshell water vapor conductance (mg d$^{-1}$ Torr$^{-1}$, where 1 Torr = 133.322 Pa) was measured during the first half of the incubation period following the method described by Tullett (1981).

The metabolic chambers (0.4 L; 20 chambers available) were ventilated with dry air. Chamber temperature was equivalent to incubation temperature. Flow rates were adjusted by a mass flowmeter (Hi-Tec F201/EB; maximum flow, 500 mL min$^{-1}$), so that O$_2$ and CO$_2$ concentrations of the dried outlet air did not drop below 20% or rise above 1%, respectively. After the eggs were put into the metabolic chambers, the system was allowed to reach equilibrium during a period of at least 60 min. Thereafter, O$_2$ and CO$_2$ concentrations of the dried outlet air from each chamber were measured continuously with a paramagnetic O$_2$ analyzer (Taylor Servomex OA184) and an infrared CO$_2$ analyzer (Heraeus Leybold Binos), respectively, until a stable registration for approximately 10 min was obtained. On average, measurements lasted 15 min per chamber. The O$_2$ and CO$_2$ concentrations of the inlet air were measured hourly. Average egg metabolic rate (M$_{egg}$; mW) was computed as: $M_{egg} = 4.49 \frac{V_{O_2}}{g} + 1.39 \frac{V_{CO_2}}{g}$, where $V_{O_2}$ is the volume of O$_2$ consumed and $V_{CO_2}$ is the volume of CO$_2$ produced, both in liters (STPD) per hour (Romijn and Lokhorst 1961). Before calculating egg metabolic rate, the O$_2$ concentrations of the outlet air were corrected for the differences in volume of the inlet and outlet air at respiratory quotients below 1.0 (Hill 1972).

Material and Methods

Eggs and Incubation

Fresh eggs of turkey (87.70 ± 0.29 g SEM; strain: B.U.T. Big 6), guinea fowl (51.20 ± 0.17 g SEM; strain: Galor), and Japanese quail (14.53 ± 0.07 g SEM; meat type) were weighed before storage at approximately 18°C for 3–5 d before the start of incubation. Turkey and guinea fowl eggs were incubated initially at 37.5°C and 37.2°C and 53% and 50% relative humidity, respectively, and automatically turned hourly. At day 25 of incubation, individual eggs were laid horizontally in mesh wire boxes and further incubated at 37.0°C and 80%–85% relative humidity (both species). Quail eggs were incubated at 37.6°C and 80% relative humidity throughout the incubation period and turned hourly automatically. At day 15, individual eggs were laid horizontally in mesh wire boxes. In all species, the eggs were no longer turned after they were put into the mesh wire boxes. After the first occurrence of external pipping, the eggs were regularly checked for hatching (approximately every 2 h during daytime and every 4 h during nighttime).
mined by extraction with petroleum ether (40°–60°C for at least 24 h). Fat-free dry mass was calculated by subtracting fat mass from total dry mass. Determinations of fat content appeared unreliable in embryos weighing less than 2 g (only quail and very young guinea fowl); these were excluded from the analysis. The amount of deposited embryonic tissue at a certain age was estimated from the difference in mean mass and time from the sample groups of the preceding and succeeding ages.

Calculation of Energy Budgets

As stated in the introduction, the daily energy expenditure of an egg could be divided into five components: (1) embryonic maintenance, (2) synthesis costs, (3) muscular activity, (4) growth and metabolism of the extraembryonic membranes, and (5) energy deposited as new embryonic tissue. Only the last component, the rate of energy deposited in the embryonic tissues $({E_{\text{em}}} \text{kJ d}^{-1})$, was easy to determine—in our case, by multiplying the amounts of fat and fat-free dry matter deposited by their energetic values (37.7 and 20.5 kJ g$^{-1}$, respectively; Ricklefs 1974): $E_{\text{em}} = 37.7 \times (\text{grams of deposited fat}) + 20.5 \times (\text{grams of deposited fat-free dry matter})$. Estimating the other four components of the energy budget was possible only if some assumptions were made.

Because embryonic activity was generally low (Hamburger et al. 1993), muscular activity costs were considered negligible. The metabolism of the extraembryonic membranes was considered negligible by using values determined for the domestic chicken (Romanoff 1967). We assumed that in all species the ratios of the metabolism of extraembryonic membranes and egg metabolic rate were equal at the same relative incubation time, and we estimated the metabolism of extraembryonic membranes ($M_{\text{memb}} \text{kJ d}^{-1}$) as follows: $M_{\text{memb}} = M_{\text{egg}} \times (M_{\text{chick}}/M_{\text{egg, chicken}})$, where $M_{\text{memb, chicken}}$ and $M_{\text{egg, chicken}}$ are the extraembryonic membrane and overall egg metabolic rate, respectively, of the domestic chicken, and $M_{\text{egg}}$ is the average egg metabolic rate of the species in question (Romanoff 1967). It was impossible to estimate the amount of energy deposited in new extraembryonic membrane tissues, because we could not separate the yolk well enough from the yolk sac membrane in our eggs, and Romanoff (1967) did not present the fat and protein content of all three membranes. Early in incubation, when most of the membrane growth occurs, the overall energy demand of the extraembryonic membranes was thus clearly underestimated. However, the energy deposited in the membranes, like the energy deposited by the embryo, is not incorporated in overall egg metabolic rate as measured by its oxygen consumption. This means that missing the energy deposited by the membranes in the overall daily energy budget will not effect the estimates of embryonic maintenance, synthesis costs, and synthesis efficiency, which are the estimates of interest here.

The last two components of the energy budget, synthesis and maintenance costs, were assessed by calculating either the synthesis costs first, referred to throughout as the “synthesis model,” or the maintenance costs first, referred to throughout as the “maintenance model.” The synthesis model is the method commonly used to calculate energy budgets in growing young animals (see, e.g., Olson 1992; Klaassen 1994). The synthesis costs $({E_{\text{syn}}} \text{kJ d}^{-1})$ were estimated as follows: $E_{\text{syn}} = E_{\text{em}} \times [(1 - e_{\text{syn}})/e_{\text{syn}}]$, where $e_{\text{syn}}$ is the synthesis efficiency and $E_{\text{em}}$ is the energy deposited in new tissue. Embryonic maintenance costs $({M_{\text{main}}} \text{kJ d}^{-1})$ can then be calculated as follows: $M_{\text{main}} = M_{\text{egg}} - E_{\text{syn}} - M_{\text{memb}}$. A problem with this model was that the embryonic synthesis efficiency was unknown. For this reason, two series of energy budgets were calculated assuming a minimal and a maximal synthesis efficiency. The minimal synthesis efficiency was assumed to be the general net synthesis efficiency of birds (0.75; Ricklefs 1974), which will most likely underestimate the embryonic synthesis efficiency and thus overestimate the synthesis costs. Therefore, these calculations represent the upper limit of the synthesis costs. We will refer to these calculations as the “minimum synthesis model.”

In the minimum synthesis model, we assumed that the synthesis efficiency remained constant throughout incubation, which might be invalid. An indication of the variation in synthesis efficiency with incubation time and its effect on the energy budget was assessed by calculating daily maximal synthesis efficiencies. Using the theoretical, maximal biochemical efficiencies of the deposition of fat from fat (0.96) and protein from protein (0.86; Blaxter 1989), we estimated the daily maximal synthesis efficiency ($e_{\text{max}}$: $e_{\text{max}} = [0.96 \times \text{(energy deposited in fat)} + 0.86 \times \text{(energy deposited in fat-free dry matter)}] / (\text{total energy in deposited tissue})$. The daily maximal synthesis efficiency will always overestimate the true synthesis efficiency and thus underestimate the synthesis costs. These calculations therefore represent the lower limit of the synthesis costs. We will refer to these calculations as the “maximum synthesis model.”

In the second model, the maintenance model, the embryonic maintenance costs were estimated first. Galliform embryos do not actively regulate their body temperature, although after internal pipping, incipient thermoregulation may develop (Whittow and Tazawa 1991). Therefore, maintenance metabolism was estimated from the allometric relationship between adult, reptilian basal metabolic rate at 38°C and body mass (Schmidt-Nielsen 1984, Table 7.5). This ambient temperature was very close to the incubation temperature, which suggests that it was appropriate to use this allometric relationship. While estimating embryonic maintenance, the major difference in water content between embryos and adults should also be taken into account. Embryonic water content exceeds that of adults and varied with incubation time (see Results). Assuming an adult reptilian water content of 70% (van Marken Lichtenbelt et al. 1993), we calculated embryonic maintenance costs in two steps. First, we calculated for the embryo a reptilian basal metabolic rate (BMR, kJ d$^{-1}$) as follows: $\text{BMR} = 86.4 \times (0.86 m^{0.75})$, where $m$ is embryonic mass (kg) and 86.4 is the conver-
sion factor to change from watts (used by Schmidt-Nielsen [1984]) to kilojoules per day. Then we calculated embryonic maintenance \( (M_{\text{main}} \, \text{kJ} \, \text{d}^{-1}) \) by correcting the result of the first step for the water content differences: \( M_{\text{main}} = m_{\text{dry}} \times (\text{BMR}/0.3m) \), where 0.3\( m \) represents dry mass based on the reptile water content and \( m_{\text{dry}} \) is the actual dry embryonic mass (kg). The synthesis costs \( (E_{\text{syn}} \, \text{kJ} \, \text{d}^{-1}) \) were then calculated as follows: \( E_{\text{syn}} = M_{\text{egg}} - M_{\text{main}} - M_{\text{membr}} \).

Thus, in the maintenance model, no assumptions were made concerning the embryonic synthesis efficiency. From the results of the maintenance model, a synthesis efficiency \( (e_{\text{syn}}) \) was calculated for each day as follows: \( e_{\text{syn}} = E_{\text{syn}}/(E_{\text{syn}}+E_{\text{main}}) \). Reasonableness of the assumptions made in the maintenance model to estimate the embryonic maintenance costs was easily checked. The maintenance and synthesis costs and daily synthesis efficiencies obtained from the maintenance model should fall within the range obtained from the minimum and maximum synthesis models, which represent upper and lower limits of the reliable range.

**Statistics**

All data are presented as mean ± SEM. Unfortunately, Romanoff (1967) presented his data normalized to an average egg of 60 g (fresh mass) and without SEM. Comparisons were made by ANOVA with SYSTAT (Wilkinson 1990).

**Results**

**Metabolic Rate and Embryonic Growth**

In all species, the increase of egg metabolic rate with incubation time reached a plateau at about 80% of the incubation period (Fig. 1). The plateau length was 4 d in turkeys and guinea fowl (in both species from day 22 to day 25; \( n = 181, F_{1,177} = 1.593, P > 0.1 \) and \( n = 162, F_{1,158} = 0.626, P > 0.5 \) for turkeys and guinea fowl, respectively) and 2 d in quail (day 14–15; \( n = 64, F_{1,63} = 0.652, P > 0.5 \)) and chickens (day 17–18). A plateau could not be detected in the development of dry embryo mass, which increased continuously with incubation time (Fig. 2). The difference in development between egg metabolic rate and dry embryo mass was more obvious when the daily increases in egg metabolic rate and dry embryo mass (i.e., growth rate) were plotted versus relative incubation time (Fig. 3A, B). Embryonic growth rate increased until about 70% of the incubation period. Thereafter, it declined slightly or was more or less stable, remaining above zero. In other words, embryo mass increased continuously throughout incubation. In contrast, the daily increase in egg metabolic rate decreased dramatically from about 70% of incubation onward, approaching almost zero during the plateau phase. The increase in embryo mass was thus not reflected in the daily increase in egg metabolic rate. At the end of the plateau phase, the rate of increase in egg metabolic rate increased rapidly, representing the hatching costs.

During incubation, embryonic water content decreased from about 93% early in incubation to a stable value of about 80%. The onset of this stable phase coincided roughly with that of the plateau in metabolic rate and ranged from day 22 to day 27 in turkeys \( (80.2\% \pm 0.2\%; n = 24, F_{1,18} = 0.788, P > 0.5) \), from day 23 to day 28 in guinea fowl \( (78.0\% \pm 0.2\%; n = 24, F_{1,18} = 2.049, P > 0.1) \), from day 14 to day 17 in quail \( (80.2\% \pm 0.3\%; n = 15, F_{1,11} = 1.139, P > 0.3) \), and from about day 18 to day 21 in chickens \( (80.5\% \pm 0.6\%; n = 4) \). During the first half of incubation, embryonic fat content was low (<20% of dry embryo mass). In the second half of incubation, the fat content increased considerably, up to about 30% of the dry mass at the end of incubation. Increasing fat content resulted from an increase in the fraction of fat deposited in new tissue, which occurred around the plateau phase in egg metabolic rate (Fig. 3C).

**Energy Budgets**

The embryos deposited high amounts of energy during incubation. After the onset of rapid embryonic growth, the energy...
Synthesis Efficiency Shifts during Embryonic Growth

Because of these high synthesis costs, embryonic maintenance costs calculated from the minimum synthesis model were low and even negative at certain days of incubation (Fig. 4A). Furthermore, the start of increase in embryonic maintenance occurred in the minimum synthesis model well after the onset of rapid embryonic growth and coincided with the onset of the plateau in metabolic rate. The maintenance costs calculated from both the maximum synthesis and maintenance models, however, increased from onset of rapid embryonic growth onward (Fig. 4B, C). This suggests that the actual embryonic synthesis efficiency must have been higher than 0.75.

Figure 4 also shows that the increase in maintenance after the plateau phase, which reflects hatching costs, was not present

deposited in new embryonic tissue increased from about equal to egg metabolic rate to two and sometimes even three times egg metabolic rate. A small change in synthesis efficiency will thus have a large impact on the synthesis costs and, consequently, on the calculated maintenance. To compare the results of the energy budgets calculated according to the two models in a simple way, values were averaged over the period between the onset of rapid growth and the first day of the plateau phase in metabolic rate and expressed proportional to egg metabolic rate (Table 1). During this period, embryo mass exceeded 50% of hatching mass, embryo growth rate was high, membrane metabolism was stable, and egg metabolic rate increased as was expected from the increasing embryonic mass. This suggests that no major changes occurred in the system that could effect the energy budgets.

The slightly different synthesis efficiencies used in the minimum and maximum synthesis models yielded strikingly different synthesis costs (Table 1). The minimal synthesis efficiency (0.75) was 0.8 times the average maximal daily synthesis efficiency (0.90 ± 0.002). As a result, the average relative synthesis costs of the minimum synthesis model (69.8% ± 3.9%) were 2.97 times those of the maximum synthesis model (23.5% ± 1.5%). Because of these high synthesis costs, embryonic maintenance costs calculated from the minimum synthesis model were low and even negative at certain days of incubation (Fig. 4A). Furthermore, the start of increase in embryonic maintenance occurred in the minimum synthesis model well after the onset of rapid embryonic growth and coincided with the onset of the plateau in metabolic rate. The maintenance costs calculated from both the maximum synthesis and maintenance models, however, increased from onset of rapid embryonic growth onward (Fig. 4B, C). This suggests that the actual embryonic synthesis efficiency must have been higher than 0.75.

Figure 4 also shows that the increase in maintenance after the plateau phase, which reflects hatching costs, was not present

Figure 2. Change in dry yolk-free embryo mass (mean ± SEM, except in chicken, because Romanoff [1967] presented only means), with relative incubation time in turkey, guinea fowl, quail, and chicken. Numbers were four eggs or hatchlings per age, except for days 9 and 15 in turkeys (n = 3) and days 6, 8, 9, 11, 12, and 16 in quail (n = 1, 1, 5, 3, 3, and 3, respectively). Filled circles, yolk-free embryo; open circles, hatching. Note that in general the SEM was very small and fell within the size range of the symbols used.

Figure 3. Variation of the increase in egg metabolic rate (A), dry embryo growth rate (B), and the fraction fat of embryonic dry mass deposited (C) with relative incubation time. The shaded bars indicate the plateau phase in egg metabolic rate.
Table 1: Results of energy budgets calculated from the maintenance and synthesis models, averaged over the period of rapid growth before the plateau phase in egg metabolic rate

<table>
<thead>
<tr>
<th>Egg Metabolic Rate (J d⁻¹)</th>
<th>Energy Deposited in New Tissue (J d⁻¹)</th>
<th>Relative Maintenance Costs (%)</th>
<th>Relative Synthesis Costs (%)</th>
<th>Relative Membrane Metabolism (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthesis Efficiency</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Minimum synthesis model</td>
<td>.75</td>
<td>9,492 (3,558)</td>
<td>22,074 (11,672)</td>
<td>13.9 (7.2)</td>
</tr>
<tr>
<td>Maximum synthesis model</td>
<td>.91 (.00)</td>
<td>9,492 (3,558)</td>
<td>22,074 (11,672)</td>
<td>64.0 (1.0)</td>
</tr>
<tr>
<td>Maintenance model</td>
<td>.78 (.02)</td>
<td>9,492 (3,558)</td>
<td>22,074 (11,672)</td>
<td>27.8 (2.6)</td>
</tr>
</tbody>
</table>

Turkey, days 15–21 (n = 5):
- Minimum synthesis model: .75, 9,492 (3,558) J d⁻¹, 22,074 (11,672) J d⁻¹, 13.9 (7.2) %, 72.3 (8.1) %, 13.8 (1.4) %
- Maximum synthesis model: .91 (.00), 9,492 (3,558) J d⁻¹, 22,074 (11,672) J d⁻¹, 64.0 (1.0) %, 22.2 (2.7) %, 13.8 (1.4) %
- Maintenance model: .78 (.02), 9,492 (3,558) J d⁻¹, 22,074 (11,672) J d⁻¹, 27.8 (2.6) %, 58.4 (1.6) %, 13.8 (1.4) %

Guinea fowl, days 15–21 (n = 5):
- Minimum synthesis model: .75, 5,748 (2,296) J d⁻¹, 11,811 (5,925) J d⁻¹, 21.6 (4.4) %, 64.4 (5.5) %, 14.1 (1.5) %
- Maximum synthesis model: .90 (.00), 5,748 (2,296) J d⁻¹, 11,811 (5,925) J d⁻¹, 64.8 (.9) %, 21.1 (1.7) %, 14.1 (1.5) %
- Maintenance model: .78 (.02), 5,748 (2,296) J d⁻¹, 11,811 (5,925) J d⁻¹, 33.0 (2.4) %, 53.0 (1.0) %, 14.1 (1.5) %

Quail, days 11–13 (n = 3):
- Minimum synthesis model: .75, 1,649 (602) J d⁻¹, 4,772 (1,594) J d⁻¹, 5.2 (17.4) %, 82.8 (16.9) %, 12.0 (1.4) %
- Maximum synthesis model: .89 (.01), 1,649 (602) J d⁻¹, 4,772 (1,594) J d⁻¹, 58.3 (8.6) %, 29.7 (7.7) %, 12.0 (1.4) %
- Maintenance model: .93 (.08), 1,649 (602) J d⁻¹, 4,772 (1,594) J d⁻¹, 72.7 (16.0) %, 15.3 (17.2) %, 12.0 (1.4) %

Chicken, days 11–16 (n = 6):
- Minimum synthesis model: .75, 5,137 (1,923) J d⁻¹, 10,742 (5,396) J d⁻¹, 17.5 (2.6) %, 65.7 (4.9) %, 16.8 (2.4) %
- Maximum synthesis model: .89 (.00), 5,137 (1,923) J d⁻¹, 10,742 (5,396) J d⁻¹, 59.6 (0.8) %, 23.6 (1.8) %, 16.8 (2.4) %
- Maintenance model: .79 (.01), 5,137 (1,923) J d⁻¹, 10,742 (5,396) J d⁻¹, 31.1 (2.0) %, 52.1 (1.4) %, 16.8 (2.4) %

Note. Two series of the synthesis model were calculated, with either a general minimum synthesis efficiency (the minimum synthesis model) or an estimated daily maximal synthesis efficiency (the maximum synthesis model). From the results of the maintenance model, daily synthesis efficiencies were calculated. Maintenance and synthesis costs and extraembryonic membrane metabolism are presented as a percentage of overall egg metabolic rate. Values are presented as means (±SEM).

Comparing the results of the maintenance and minimum synthesis models revealed that the average synthesis efficiency calculated from the maintenance model was slightly higher than the minimal synthesis efficiency (1.04 times), while the relative synthesis costs were only 0.8 times those calculated from the minimum synthesis model. In other words, in the plateau phase (Fig. 5C). During the hatching period, the maintenance model thus underestimated the synthesis costs and underestimated the synthesis efficiency.

During the period of rapid embryonic growth, the average synthesis efficiency from the maintenance model fell between the minimal value (0.75) and the average maximal daily synthesis efficiency, with the exception of quail, where the estimated synthesis efficiency exceeded the average maximal daily synthesis efficiency (Table 1). Since the actual synthesis efficiency cannot exceed the maximal daily synthesis efficiency, the results from the maintenance model were considered to be unreliable in quail and were excluded from further analysis. The average estimated synthesis efficiency did not differ between the three remaining species (overall synthesis efficiency: 0.78 ± 0.01; n = 16, F₂,13 = 0.065, P > 0.9) and was significantly higher than 0.75. The average relative synthesis costs were slightly, but significantly, higher in turkey than in guinea fowl and chicken (Table 1; n = 16, F₂,13 = 6.129, P < 0.02). We nevertheless combined these three species to compare the results with the other models.

Mass-Specific Maintenance and Synthesis Costs

Using embryonic dry mass and dry growth, we were able to calculate mass-specific maintenance and synthesis costs. As explained earlier, the results from the maintenance model should fall within the range set by the results from the minimum and maximum synthesis models, which represent the upper and lower limits of the synthesis and maintenance costs.
incubation time differed between the three models (Fig. 6B). In the minimum synthesis model, where the synthesis efficiency used was constant, the mass-specific synthesis costs increased around the plateau phase in egg metabolic rate. This increase reflects the increase in the energy deposited due to the increasing fat fraction in deposited tissue (Fig. 3C). In the maximum synthesis model, daily maximal synthesis efficiencies were estimated by using the composition of tissue deposited. The maximal synthesis efficiency increased slightly around the plateau phase (Fig. 7A), as fat deposition has a higher theoretical synthesis efficiency than protein. Because of this slight increase in synthesis efficiency, the mass-specific synthesis costs from the maximum synthesis model remained constant throughout incubation (Fig. 6B). The maintenance model overestimated the synthesis costs and underestimated the synthesis efficiency dur-

In all models, mass-specific maintenance decreased rapidly with incubation time initially, reaching its lowest level at about 80% of incubation time, that is, at the onset of the plateau in metabolic rate (Fig. 6A). Thereafter, mass-specific maintenance remained more or less stable. Hatchling mass-specific metabolic rate was much higher than mass-specific embryonic maintenance just before hatching, because hatchlings are achieving homeothermy, and their metabolic rate may also include costs of biosynthesis. The mass-specific maintenance costs from the maintenance model were not out of the range set by the two synthesis models, which suggests that the estimate used for maintenance, reptilian basal metabolic rate, may have been appropriate.

In contrast to mass-specific maintenance, the shape of the relationships between mass-specific synthesis costs and relative
Discussion

By calculating daily energy budgets of developing eggs and their components and by varying the assumptions used in the calculations, it is possible to gain insight not only into the energetic costs of embryonic maintenance and growth but also into the underlying physiological processes. However, as all measurements have to be made on different individual eggs, care should be taken that factors affecting metabolic rate and embryo mass, such as egg mass and eggshell water vapor conductance, are controlled for as much as possible. As shown, results from the models in which the synthesis costs were estimated with an assumed synthesis efficiency were very sensitive to the value of synthesis efficiency used (minimum and maximum synthesis models). Therefore, we also have calculated energy budgets according to a model in which no assumptions were made on the synthesis efficiency, but which was affected by the estimate of maintenance costs (maintenance model).

We estimated embryonic maintenance from the allometric relationship for adult reptilian basal metabolic rate at $38^\circ C$ and corrected for the differences in water content between adult reptiles and galliform embryos. The results show that this estimate may not have been entirely valid, especially early and late in incubation. Problems could lie with the allometric relationship itself or with the adult reptilian water content estimation,

![Figure 6](image1.png)

Figure 6. A, Variation of mass-specific maintenance with relative incubation time. B, Variation of the synthesis costs per gram of embryo dry tissue with relative incubation time. In both panels, the results for all species are presented, except for the maintenance model, where the quail is excluded (see Results).

ing hatching (see previous section). Therefore, mass-specific synthesis costs or synthesis efficiencies calculated from the maintenance model are not presented for this period. Mass-specific synthesis costs from the maintenance model decreased initially with incubation time, reaching lowest values around the onset of the plateau phase in metabolic rate (Fig. 6B). The estimated synthesis efficiency from maintenance model showed a reversed relationship with incubation time; it increased with incubation time initially toward the highest values at the plateau phase (Fig. 7B). The mass-specific synthesis costs from the maintenance model exceeded levels from the minimum synthesis model early in incubation, which suggests that the results of the maintenance model were not reliable early in incubation.

![Figure 7](image2.png)

Figure 7. A, Variation of the maximal synthesis efficiency with relative incubation time. B, Variation of the estimated synthesis efficiency with relative incubation time. This synthesis efficiency was calculated from the maintenance model (see Material and Methods). In both panels, the shaded bar indicates the plateau phase in egg metabolic rate.
or they could also be caused by changes in the maturity of the embryo.

For both models, a problem may also be found in the estimation of the metabolic rate of the extraembryonic membranes. Ar et al. (1987) measured metabolic rate of the chorioallantoic membrane and found an oxygen consumption that was twice that given by Romanoff (1967), the value used here. If this difference applies to all extraembryonic membranes, the membrane metabolism component doubles. This will result in a decrease in maintenance costs when using our synthesis models and a decrease in synthesis costs when using our maintenance model. As a result, energy budgets from the maintenance model will approach the results from the maximum synthesis model.

To explain the paradox of the plateau phase of metabolic rate, the changes in the different components of the energy budget during incubation should be followed. Mass-specific maintenance was high early in incubation. This is probably mainly due to the cell and organ differentiation that occurs early in incubation. In domestic chicken embryos, the preliminary brain and heart structure and the gut with its initial connection to the yolk are completed at day 5 of incubation (Romanoff 1960). With the completion of preliminary organ structures, it is possible that the costs of differentiation may decrease, resulting in a decrease in mass-specific maintenance. From the plateau onward, mass-specific maintenance remains stable (Fig. 6A). Precocial hatchlings possess functional sensory, neuromuscular, and thermoregulatory systems (Whittow and Tazawa 1991). Vleck et al. (1979) suggested that maturation of these systems especially occurs at the end of incubation. This increased maturity, together with the increasing activity costs due to the hatching process, is expected to induce an increase in mass-specific maintenance at the end of incubation. The results show, however, no indication of this expected increase. Note that an increase in mass-specific maintenance may be very unfavorable during the plateau phase in metabolic rate, as the embryo continues to grow but apparently cannot increase its oxygen consumption, unless it can tolerate a lower blood oxygen tension and a higher carbon dioxide tension (Rahn 1981; Okuda and Tazawa 1988).

At the onset of the plateau phase, mass-specific maintenance reaches its lowest level and probably remains at that level thereafter. Because embryo mass continues to increase during the plateau phase, overall maintenance costs will increase. As egg metabolic rate remains constant, another component of the energy budget is expected to decrease to balance the energy budget, for example, the synthesis costs. The results of the maintenance model, showing a decrease in mass-specific synthesis costs during the plateau, indicate that this might occur. A decrease in synthesis costs can be achieved in several ways. First, the synthesis costs may decrease by decreasing (slightly) the amount of tissue deposited during the plateau phase, such as is found in the turkey (Fig. 3B). Second, the synthesis costs will decrease rapidly if the synthesis efficiency increases. In postnatal and adult mammals and birds, the synthesis efficiency varies with age, temperature, composition of diet, and type of substance accumulated (Ricklefs 1974). The increase in the ratio between deposited fat and fat-free dry matter around the plateau phase (Fig. 3C) will probably increase the synthesis efficiency (Blaxter 1989). Furthermore, the synthesis efficiency may increase from a change in the substrate used to synthesize fat (Ricklefs 1974). The synthesis efficiency of fat is highest when fat is used as a substrate (Blaxter 1989). Our data do not allow a reliable indication of the substrates used for fat deposition. However, at the end of incubation, selective uptake of yolk fat occurs (M. W. Dietz, unpublished data), which suggests that at least during the plateau phase, fat was deposited directly from fat absorbed from yolk.

Apart from decreasing the synthesis costs, the embryo has other possible ways to change the allocation of energy in favor of maintenance. One is a decrease in activity. Activity costs were neglected in the calculation of the energy budgets, and estimation is troublesome. Spontaneous muscular activity begins early in the ontogeny of the chicken embryo (days 4–5) and increases during development (Hamburger 1963). At the last day of the plateau phase, the chicken embryo changes its position in the egg into the “hatching position” (Hamburger and Oppenheim 1967). This process may take several hours, and the amount of spontaneous activity occurring besides the position change does not change much. Therefore, it seems unlikely that the activity costs at the last day of the plateau were lower than at the first day.

Another possibility involves the formation of oxygen-rich metabolites such as glycogen. Glycogen is a major energy source during pipping, and it is used in anaerobic glycogenolysis (Freeman 1969). During the formation of glycogen, oxygen is chemically bonded to oxygen-poor metabolites, as suggested by low respiratory quotient values (Beattie 1964; Freeman 1969). Before the plateau phase, glycogen is formed and stored in the liver. At the beginning of the plateau phase, this process ends (Freeman 1969). A higher atmospheric concentration of oxygen (23.5%) during the plateau phase or removal of the eggshell at the air cell increases the amount of glycogen stored in the liver at the end of the plateau phase (Beattie 1964; Christensen and Donaldson 1992). This suggests that the formation of glycogen is depressed during the plateau phase, so that more oxygen is available for other processes. However, the significance of this process cannot be evaluated, as the amount of oxygen involved in glycogen formation could not be separated from the maintenance component.

Finally, anaerobic metabolism could occur, but this is not likely because the energetic output of anaerobic metabolism is much lower than that of aerobic metabolism (Blaxter 1989). Also, the concentration of lactate in blood plasma in chicken embryos does not increase before internal pipping (Tazawa et al. 1983).

In summary, during the plateau phase an embryo may change its energy allocation in favor of maintenance costs in three ways: a decrease in growth rate, an increase in the synthe-
sis efficiency, and a decrease in the formation of glycogen. Our results for Galliformes indicate that an increase in synthesis efficiency, rather than a reduction in growth rate, may explain the plateau paradox. A small increase in synthesis efficiency can lead to a tremendous decrease in synthesis costs owing to the high embryonic growth rates.

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