Oxygen Transport in the Avian Egg at High Altitude

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SYNOPSIS. Oxygen transport to avian embryo tissues occurs by three steps, two of which are driven by diffusion. This results in a series of stepwise decrements in $P_O_2$ between atmosphere and tissue. The $P_O_2$ decrements for embryos of the domestic fowl incubated at different altitudes are used here to examine potential adaptations to hypobaric hypoxia. With exposure to moderate hypoxia embryos of the domestic fowl appear to maintain adequate tissue oxygenation. Adaptive adjustments in the shell, shell membranes and chorioallantois complex were not observed. However, hemoglobin-O$_2$ affinity was increased and preliminary evidence suggests a redistribution of blood flow to maintain adequate oxygenation in higher priority areas of embryonic tissue. At severe hypoxia, embryos of the domestic fowl show decreased $O_2$ consumption, embryo mass and lengthened incubation period. Thus, at severe hypoxia, the embryo of the domestic fowl does not appear to provide a realistic model. Evidence from avian embryos of species native to high altitude suggest that they are able to maintain adequate tissue oxygenation even at severe hypoxia. Preliminary evidence suggests that some of the blood, vascular system and tissue level adaptations present in the chicken embryo are also present in species native to high altitude. One of these, an increase in embryonic hemoglobin-O$_2$ affinity which is physiologically mediated in the chicken embryo is genetically-based in the embryo of the native high-altitude species.

INTRODUCTION

Birds are the most successful vertebrate class in terms of survival and reproduction at high altitude. At least 12 species have been reported breeding near or above 5000 meters, and one species, the alpine chough (*Phyrrocorax graculus*) has been found nesting as high as 6500 meters (Rahn, 1977). The partial pressure of oxygen ($P_O_2$) at this altitude is approximately 68 torr, 43% of sea level $P_O_2$.

It has been suggested that animal species native to altitude have successfully solved the problems related to the constraints imposed by hypobaric hypoxia and that the upper limit for their distribution is determined by the availability of food (Morrison, 1964). However, comparatively little information has accumulated which directly answers the question of how avian embryos developing at extreme altitude are able to transport adequate $O_2$ to the tissues.

In this paper we will describe the experimental evidence, both direct and indirect, which addresses this question. Investigations on eggs from the domestic fowl, the most thoroughly examined species, and selected wild species will be reviewed. We will attempt to identify points in the $O_2$ transport pathway at which physiological and/or structural adaptations to hypoxia may be present.

DOMESTIC FOWL

The chain of oxygen supply

Oxygen transport from the nest to the tissues follows a series of stepwise decrements in $P_O_2$. Each of the decrements can be viewed as a link, and the sum of the links forms the chain of $O_2$ supply to the tissues (Hurtado, 1964; Metcalfe, 1969/1970). For the avian embryo, the chain may be divided into three steps: 1) the diffusion of $O_2$ through the shell, two underlying shell membranes, and chorioallantoic...
membrane, 2) the convective mass transport of $O_2$-rich blood, and 3) the diffusion of $O_2$ from the blood to the embryonic tissues (see Tazawa, 1980, for a development of $O_2$ transport). Since two of the three steps depend upon diffusion and thus are driven by a $P_{O_2}$ gradient, any factor which increases $P_{O_2}$ at any point in the $O_2$ transport chain will serve to improve tissue oxygenation (Metcalfe, 1969/1970). Oxygen partial pressures under normoxia (sea level, nest $P_{O_2}$ = 150 torr), moderate hypoxia (1500 meters, nest $P_{O_2}$ = 125 torr) and severe hypoxia (3800 meters, nest $P_{O_2}$ = 95 torr) for the various points in the $O_2$ transport chain for the 18-day chicken embryo are modelled in Figure 1 and discussed below.

**Normoxia**

The only condition for which there is nearly complete information for all levels in the $O_2$ transport chain is normoxia (Fig. 1). Using this information, the major links in the $O_2$ supply chain, and thus the areas which offer the greatest potential for adjustments to hypoxia can be considered.

*Shell, shell membranes, and chorioallantois.*

The difference in $P_{O_2}$ at each step in the $O_2$ transport chain reflects the relative $O_2$ diffusion resistance of each of the structures interposed between atmosphere and blood. When viewed in the context of adaptation to altitude, consideration of the resistances of each of these structures becomes significant, since reductions in diffusion resistance will increase tissue $P_{O_2}$.

Published values for $O_2$ conductance (the inverse of resistance) through dry scoured shell and shell plus dry membranes (Tullett and Board, 1976), moist inner and outer shell membranes (Kutchai and Steen, 1971), and in vivo inner membrane plus chorioallantois (Piiper et al., 1980) have been used to calculate diffusion resistance values appropriate for the 18-day embryo (Table 1). These calculated values, although derived from a number of different sources, sum to a total which is consistent with the total $O_2$ diffusion resistance between atmosphere and blood calculated from the $D_{O_2}$ measurements of Temple and Metcalfe (1970).

In the egg of the 18-day domestic fowl the shell is a major site of resistance to the diffusion of $O_2$ (Table 1). The outer shell membrane offers negligible resistance (Lomholt, 1976; Paganeli et al., 1978). The resistance of the inner shell membrane has not been determined with certainty, however. Kutchai and Steen (1971), Tullett and Board (1976) and Lomholt (1976) have suggested that the inner shell membrane provides a relatively great resistance. In contrast, Rahn et al. (1979) imply that it offers negligible resistance. Rahn et al. (1979) have published an electron photomicrograph suggesting the presence of a "film" between the inner shell membrane and the chorioallantois. They suggest that the film could contribute to the high resistance in this region. Since quantitative estimates of the relative importance of the film are lacking, we have assigned all of the resistance in this area, 32%, to the inner shell membrane. The final resistance to the diffusion of oxygen into the blood is the chorioallantois which accounts for 26% of the total resistance. The chorioallantois is the most complex layer since resistance at this level may vary not only with chorioallantoic area (Roman-
O₂ Transport in Eggs at High Altitude

off, 1960) and thickness of the layers between capillary lumen and the inside of the inner shell membrane (Duncker, 1978), but with capillary density, blood hemoglobin concentration and the rate of blood flow as well (Tazawa and Ono, 1974).

Blood. During the last half of incubation, blood O₂ capacity increases dramatically (see review by Tazawa, 1980). In spite of this, the Po₂ in arterialized blood falls, from 97 torr on day 9 (Tazawa, 1971) to 52 torr on day 19 (Freeman and Misson, 1970). Arterial O₂ saturation during this period remains high at about 80-90% (Tazawa and Mochizuki, 1977) due to a progressive increase in hemoglobin-O₂ affinity (Bartels et al., 1966; Farooqui and Huehns, 1972; Misson and Freeman, 1972; Tazawa et al. 1976; Baumann and Baumann, 1978). Since more than 80% of the O₂ is removed from hemoglobin by some embryonic tissues on each pass through the embryonic circulation (Tazawa, 1978), the adjustment in hemoglobin-O₂ affinity is critical to maintaining adequate O₂ transport. Recent work has shown that the changes in hemoglobin-O₂ affinity are mediated by changes in intraerythrocyte ATP concentration (Misson and Freeman, 1972; Isaacks et al., 1976).

Vascular bed and embryonic tissue. The Po₂ gradient between blood in the tissue capillaries and sites of O₂ utilization in the tissues will depend in part upon the capillary density and presence of tissue myoglobin. Information on both of these areas is currently lacking, but should prove interesting, since tissue Po₂ falls to very low values as the embryo approaches hatching even under normoxic conditions (Tazawa and Mochizuki, 1977; Tazawa, 1978). Mixed venous Po₂ is approximately 20 torr in the normoxic 18-day chicken embryo (Freeman and Misson, 1970). This value should be an approximate index to the average tissue Po₂ (Tenney, 1974), but because of the relative distribution of arterialized blood to different tissues varies (Tazawa and Mochizuki, 1977; Tazawa, 1978), regional tissue Po₂ may be much lower. The Po₂ in hind limb capillaries has been estimated at about 5 torr (Tazawa, 1978), a value which is near the minimum Po₂ required to maintain oxidative metabolism in isolated mitochondria (Chance, 1957). At this tissue Po₂ the total Po₂ gradient for the normoxic 18-day chicken embryo is 145 torr (Fig. 1).

Moderate hypoxia

We have recently studied the developing chicken embryo at moderate hypoxia (1500 meters; nest Po₂ = 125 torr), and our findings suggest that these embryos exhibit physiological adjustments which ensure normal tissue oxygenation. For example, both the growth rates and O₂ consumption rates for our embryos (Grey Leghorn strain) are the same under sea level and moderate hypoxia (Fig. 2A, B). Air cell Po₂ shows a reduction from ambient comparable to that for the normoxic embryos (Fig. 2C). Direct information on the Po₂ gradient between air cell and blood is not available, but Tazawa et al. (1971) and Metcalfe et al. (1979) have shown that eggs of chicken embryos subjected to hypoxia by covering a portion of the shell surface did not show any decrease in O₂ diffusion resistance between ambient air and blood, suggesting that O₂ transport to this level is maximized in the sea-level chicken embryo (Bissonnette and Metcalfe, 1978; Metcalfe et al., 1979). Thus, for the embryos under conditions of moderate hypoxia, the ambient air-to-blood Po₂ gradient is as great as that for normoxic embryos (Fig. 1). This suggests that arterial Po₂ must be lowered in embryos developing under moderate hypoxia. Since these embryos

<table>
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<tr>
<th>Structure</th>
<th>Calculated resistance (sec • lorr/cm)</th>
<th>% of total</th>
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<tbody>
<tr>
<td>Dry scoured shell</td>
<td>0.40 × 10⁶</td>
<td>40</td>
</tr>
<tr>
<td>Outer membrane</td>
<td>0.02 × 10⁶</td>
<td>2</td>
</tr>
<tr>
<td>Inner membrane</td>
<td>0.32 × 10⁶</td>
<td>32</td>
</tr>
<tr>
<td>Chorioallantois</td>
<td>0.26 × 10⁶</td>
<td>26</td>
</tr>
<tr>
<td>Totalb</td>
<td>1.00 × 10⁶</td>
<td></td>
</tr>
</tbody>
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a Values calculated from published permeability measurements (References in text).
b Total ambient to blood O₂ diffusion resistance calculated from Dco (Temple and Metcalfe, 1970) = 1.2 × 10⁶.
are able to maintain rates of growth and metabolism comparable to the normoxic embryos, adjustments at the blood, circulatory system, and/or tissue level are indicated.

Preliminary studies on the blood of the embryos exposed to moderate hypoxia suggest that an increased hemoglobin-O$_2$ affinity may be important. During the latter part of incubation, our embryos developing under moderate hypoxia show a hemoglobin-O$_2$ affinity which is higher than the values obtained for embryos exposed to normoxic conditions (Fig. 3A). Intraerythrocyte ATP concentrations are decreased (Fig. 3B), and the correlation between ATP and hemoglobin-O$_2$ affinity appears to remain consistent for the normoxic versus hypoxic embryos (Fig. 3C). This suggests that the hypoxic effect on hemoglobin-O$_2$ affinity is due to a difference in intraerythrocyte ATP and not to a difference in hemoglobin type (see also Atherton and Timiras, 1970).

The relative distribution of blood flow to embryonic tissues may be affected by exposure to hypoxia. For example, in our laboratory newly hatched chicks from eggs incubated at an ambient Po$_2$ of 125 torr had body weights similar to chicks incubated at an ambient Po$_2$ of 109 torr, but mean heart mass for the more hypoxic group was increased by 14% while gastrocnemius muscle mass was decreased by 26% (Table 2) (Lucich and Birchard, unpublished observations). These data are suggestive of at least two possible adjustments at the vascular bed and tissue level: 1) preferential redistribution of blood flow to areas with higher priority than the hind limb (e.g., heart and nervous system) or 2) a decrease in hind limb Po$_2$ sufficient to reduce development of tissue in this region.
Adaptations at the level of the vascular bed and tissues have not been explored in the avian embryo developing under moderate hypoxia. However, adjustments at both levels have been reported for adult and embryonic mammals exposed to hypobaric hypoxia (Becker et al., 1955; Valdiva, 1958; Bullard, 1972; Lenfant, 1973; Lechner, 1976). Thus, both areas should provide interesting focal points for future studies on the avian embryo.

**Severe hypoxia**

Considerable information is available on adjustments in the chicken embryo developing under severe hypoxia (3800 m, nest \( P_{O_2} = 95 \) torr). As with eggs laid under conditions of moderate hypoxia, air-cell \( P_{O_2} \) is decreased (Wangensteen et al., 1974). However, in contrast to embryos which develop under conditions of moderate hypoxia, incubation period is lengthened (Smith et al., 1969) and egg mass reduced, with a concomitant reduction in embryonic metabolism (Wangensteen et al., 1974; Beattie and Smith, 1975).

The reduced embryonic metabolic rate serves to decrease the \( P_{O_2} \) gradient between ambient air and embryonic tissue at each link in the \( O_2 \) transport chain (Fig. 1). This is consistent with the reported air cell values of Wangensteen et al. (1974). Whether additional adjustments occur elsewhere along the chain of \( O_2 \) supply is not known. Wangensteen et al. (1974) suggest that the reduced metabolic rate is sufficient to account for all observed differences between hypoxic and normoxic embryos, but \( O_2 \) consumption studies on these embryos suggest that they are better able to maintain weight-specific metabolic rate than are the sea-level embryos acutely exposed to hypoxia (Beattie and Smith, 1975). Thus, adaptations at the blood, vascular system, and/or tissue level may be present.

The chicken eggs described above (Smith et al., 1959; Smith, 1973) were from 12 generations of continuous breeding at the Barcroft High Altitude Laboratory over a 15 year span. The first generation showed hatchability of 16%; by generation 8, this had increased to 60%. It did not rise above this level with subsequent generations; thus, adjustment to severe hypoxia was less than complete.

**Eggs of Bird Species Native to Altitude**

The chicken embryo at high altitude appears to represent a model of limited value in terms of chronic adaptations to severe hypobaric hypoxia. Bird species native to high altitude do appear to possess adaptations to maintain adequate tissue oxygenation to severe hypobaric hypoxia. For example, Verbeek (1967) and Conry (1978) in studies on horned larks (*Eremophila alpestris*) at 2400 m and 3800 m respectively found no difference in hatchability, incubation period, or hatchling weight between their populations and a sea-level population (Drury, 1961). Although these observations are consistent with the theory that these species have adaptations which tend to maintain adequate tissue oxygenation, the mechanisms involved are completely unknown.

Recently we have examined \( O_2 \) transport in eggs of the bar-headed goose (*Anser indicus*), a native high-altitude species which nests in large numbers up to 5500 m.
(Würdinger, 1973), and Canada geese (Branta canadensis), a species native primarily to sea level (Snyder, Black, and Birchard, unpublished observations). Although this investigation is still in its early stages, some preliminary statements can be made about O₂ transport in embryos of these species at moderate hypoxia. Eggs of both species show levels of O₂ consumption which are similar, but calculated resistance of the shell to O₂ is approximately 25% greater in bar-headed goose eggs. This is reflected in pre-internal pipping air-cell gas tensions (Table 3).

Embryos from both species show similar hemoglobin levels throughout incubation, and both species show the same pattern of increase in hemoglobin-O₂ affinity during incubation followed by a decrease after hatch (Fig. 4). However the P₅₀ values for the bar-headed goose embryonic and gosling blood are consistently below those of Canada goose embryos and goslings at comparable stages of development (Fig. 4). At this point we do not have sufficient information to determine whether this difference is mediated by intraerythrocyte ATP or by intrinsic differences in hemoglobin type. It seems likely, however, that some mechanism other than ATP levels account for these P₅₀ differences, since adult bar-headed geese also have hemoglobin-O₂ affinity greater than that of sea-level waterfowl (Black et al., 1978; Black and Tenney, 1980), and this difference is not due to differences in ATP (Petschow et al., 1977).

Although studies on embryos of altricial birds have provided indirect evidence suggesting that avian embryos at high altitude are able to maintain adequate tissue Pₒ₂, problems of tissue oxygenation at high altitude may be particularly great in eggs of smaller species, most of which are altricial. This is suggested by prehatching air-cell Pₒ₂ values for two altricial species, calculated by Vleck et al. (1979) at 72 and 85 torr, considerably lower than the average of 94 torr calculated by Rahn et al. (1974) for 13 precocial and 1 altricial species. The reason for these low prehatching air-cell Pₒ₂ values may be related to the pattern of O₂ consumption just prior to hatching (Hoyt et al., 1978; Vleck et al., 1979) or to the higher-than-predicted shell resistance which appears to be present in eggs weighing less than 4-5 grams (Snyder, 1978).

CONCLUSIONS

To date, investigations into O₂ transport in the avian embryo have centered around a model based on the domestic fowl egg, although enough is now known about O₂ transport in eggs of species native to high altitude to begin to evaluate the limitations of this approach. Embryos of the domestic fowl show adjustments which appear to maintain adequate tissue oxygenation with.

**TABLE 3. Air-cell gas tensions (torr) for eggs of domestic fowl, Canada geese, and bar-headed geese just prior to internal pipping.**

<table>
<thead>
<tr>
<th>Species</th>
<th>Pₒ₂</th>
<th>Pₒ₂₂</th>
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<tbody>
<tr>
<td>Gallus gallus</td>
<td>93</td>
<td>28</td>
</tr>
<tr>
<td>Branta canadensis</td>
<td>93</td>
<td>27</td>
</tr>
<tr>
<td>Anser indicus</td>
<td>86</td>
<td>33</td>
</tr>
</tbody>
</table>

* Eggs were laid and artificially incubated at 1500 m.
exposure to moderate hypoxia (PO₂ = 125 torr). These adjustments appear as 1) an increased hemoglobin-O₂ affinity to allow blood O₂ saturation to remain high in the face of lowered arterial PO₂ and 2) possible redistribution of arterialized blood to maintain adequate tissue PO₂ in some high priority areas. Adjustments in the shell, shell membrane, chorioallantois complex which reduce O₂ diffusion resistance are not evident.

At severe hypoxia (PO₂ = 95 torr), the chicken embryo does not appear to provide a realistic model. At this PO₂, the chicken embryo is not able to maintain normal O₂ consumption or development rate, whereas in species native to this altitude, both embryonic O₂ consumption and development rate are normal.

At least some of the blood, vascular system and tissue level adaptations present in the chicken embryo also appear to be present in species native to high altitude. One of these, the increase in embryonic hemoglobin-O₂ affinity which appears to be physiologically mediated in the chicken embryo, has been translated into a genetically-based adaptation in the embryo of the native high-altitude species.

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