Ventilatory chemosensitivity of the 1-day-old chicken hatchling after embryonic hypoxia

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Szdzuy K, Mortola JP. Ventilatory chemosensitivity of the 1-day-old chicken hatchling after embryonic hypoxia. Am J Physiol Regul Integr Comp Physiol 293: R1640–R1649, 2007. First published August 8, 2007; doi:10.1152/ajpregu.00422.2007.—We investigated the effects of sustained embryonic hypoxia on the neonatal ventilatory chemosensitivity. White Leghorn chicken eggs were incubated at 38°C either in 21% O2 throughout incubation (normoxia, Nx) or in 15% O2 from embryonic day 5 (hypoxia, Hx), hatching time included. Hx embryos hatched ∼11 h later than Nx, with similar body weights. Measurements of gaseous metabolism (oxygen consumption, V˙O2) and pulmonary ventilation (V˙E) were conducted either within the first 8 h (early) or later hours (late) of the first posthatching day. In resting conditions, Hx had similar V˙O2 and body temperature (Tb) and slightly higher V˙E and ventilatory equivalent (V˙E/V˙O2) than Nx. Ventilatory chemosensitivity was evaluated from the degree of hyperpnea (increase in V˙E) and of hyperventilation (increase in V˙E/V˙O2) during acute hypoxia (15 and 10% O2, 20 min each) and acute hypercapnia (2 and 4% CO2, 20 min each). The chemosensitivity differed between the early and late hours, and at either time the responses to hypoxia and hypercapnia were less in Hx than in Nx because of a lower increase in V˙E and a lower hypoxic hypometabolism. In a second group of Nx and Hx hatchlings, the V˙E response to 10% O2 was tested in the same hatchlings at the early and late hours. The results confirmed the lower hypoxic chemosensitivity of Hx. We conclude that hypoxic incubation affected the development of respiratory control, resulting in a blunted ventilatory chemosensitivity.

IN MAMMALS AND BIRDS, the development of the respiratory system is a long process. It begins early during gestation or incubation, when the embryo’s gas-exchange needs are provided by nonpulmonary organs, and continues far into postnatal life, when pulmonary ventilation is the only means of fulfilling the aerobic requirements of the growing organism. A question of both biological and clinical interest is whether or not this process is strictly under genetic control or can be modulated by external events. In this latter case, the rather long time for structural and functional development gives the respiratory system the opportunity of adjusting to the postnatal changes in metabolic needs. Equally, however, it opens a potentially risky time window for environmental factors to interfere negatively with the normal development of the mechanisms of respiratory control, including ventilatory chemosensitivity.

Several studies have questioned whether the ventilatory responses to hypoxia or hypercapnia reflect a genetically controlled and fixed program or are a phenotypic trait that can be modified through experiences, a phenomenon often referred to as developmental plasticity (18; see Ref. 7 for review). For example, studies on kittens and rats have indicated that sustained changes in the inspired oxygen pressure, either hypoxia (17, 19, 20) or hyperoxia (3, 13, 30, 31, 36), in the neonatal period have resulted in long-lasting effects on the control of breathing, specifically in a reduction in the hypoxic ventilatory response. Evidence for developmental plasticity of the respiratory control system also comes from studies on humans born and living at high altitude, which exhibit a blunted ventilatory response to hypoxia compared with sea-level natives (16, 41).

Almost all studies performed in mammalian species have focused on the effects of chronic hypoxia during the neonatal period, whereas prenatal hypoxia exposure has received very little attention. After gestation in hypoxia, 1-day-old rats presented some respiratory and metabolic disturbances (12). In another study, after hypoxic gestation, rats at 3 wk of age had a marked augmentation of the ventilatory response to hypoxia, absent hypoxic hypometabolism, and a decreased carotid body dopamine content (32). Differently yet, rats born and raised for two generations at high altitude responded to hypoxia in the same way as sea level controls did (39). The interpretation of these results in mammals, however, is complicated by the fact that the ventilatory and hormonal responses of the mother to hypoxia during gestation have an impact on fetal development (9, 14, 23, 42). On the other hand, the avian model, in which the chorioallantoic membrane provides gas exchange, circumvents these interpretative issues, and the neonatal outcome of embryonic hypoxia can be evaluated free from the confounding effects of the maternal response. Despite the structural differences, birds and mammals have numerous similarities in their ventilatory responses to altered gaseous environments and in the control mechanisms behind these responses (2, 5, 38). Zebra finches and quails exposed to elevated CO2 during the embryonic period had some alterations in the acute ventilatory response to CO2 as adults (2, 43). Equivalent experiments with embryonic hypoxia are not available. Therefore, the primary aim of the current study was to explore the effect of sustained prenatal hypoxia on the neonatal ventilatory chemosensitivity. To this end, we exposed chicken embryos to hypoxia and tested the ventilatory responses of the hatchlings to acute hypoxia, hypercapnia, and hyperoxia.

MATERIALS AND METHODS

Freshly laid fertilized eggs of White Leghorn chickens (Gallus gallus) were obtained from a local supplier. The eggs were weighed and placed in incubators (Hova-Bator, Savannah, GA), starting around

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midday (day 0). The incubators maintained a steady temperature (T) of ~38°C and 60% relative humidity, with 45-degree egg rotation four times a day. At first, all eggs were incubated in normoxic conditions to ensure a normal development during the first critical days. Then, at embryonic day 5, they were separated into two groups. Some continued in normoxia (21% O2; Nx); others were transferred into a hypoxic incubator kept at 15–16% O2 (on average 15.3 ± 0.04%; Hx), where they remained, hatching time included, until the time of the measurements. The desired level of hypoxia was obtained by leaking a small stream of warmed and humidified N2 into the incubator from a pressurized tank under the control of a flowmeter. The O2 concentration within the incubator was continuously sampled by a calibrated fuel cell gas analyzer (FOXBOX; Sable Systems International, Las Vegas, NV) and was displayed on a computer monitor. Incubation T and relative humidity were monitored by a data logger and a hygrometer placed inside the incubator. The former collected the T data every 10 min, whereas humidity was read daily.

On the day of hatching (H1), body weight and posthatching hours were noted. Then the main protocol consisted in measuring gaseous metabolic and pulmonary ventilation during air breathing and during exposure to hypercapnia, hypoxia, or hyperoxia, either within the first 8 h (early) or later during the day (late).

**Pulmonary ventilation.** The breathing pattern was measured with an adaptation of the barometric technique originally proposed by Drorbaugh and Fenn (8). The approach stands on the fact that, when an animal is breathing inside a sealed chamber, the air inspired is warmed and humidified from the ambient to the pulmonary values, raising the chamber pressure; the opposite occurs in expiration. Since its original presentation, the technique has been modified and adapted to multiple applications (reviewed in Ref. 26), including the chick embryo (21, 22). Details of the methodology, possible problems, and validation have been discussed elsewhere (40). Briefly, the animal chamber was separated into two sections, a smaller animal compartment, acting as a "nest," of ~100 ml, where the hatching was positioned, and a larger outer compartment of ~200 ml. This separation was required to maintain the hatching at its customary T, ~37.5°C, kept constant by circulating water from a servo-controlled water bath. The nest T was monitored by a transmitter powered by a constantan thermocouple, either in a separate group of hatchlings or in the same animals before and at the end of the experiment, to avoid disturbances during the recordings.

Three polyethylene tubes passed through the lead of the chamber. Two of these lines were for continuous flushing with the desired gas mixtures. The third line was for the recording of the pressure oscillation (P) related to breathing, via a sensitive pressure transducer. The inflow line was connected to air or to a gas-impermeable 10-liter bag for the delivery of hypoxic (10 or 15% O2), hypercapnic (2 or 4% CO2), or hyperoxic (85% O2) mixtures. These gas mixtures were prepared by blending the appropriate pure gases from pressurized tanks and checking their final concentration with calibrated gas analyzers. The outflow line was connected to a suction pump, which maintained a steady flow of 150 ml/min, under the control of a precision needle-valve flowmeter. All signals (chamber and nest T, relative humidity, P and flow, O2 and CO2 concentrations) were converted digitally, acquired online by a minicomputer at a sampling rate of 100 Hz, and displayed breath-by-breath on a computer monitor. The volume calibration (K) of the chamber was obtained by injecting a known volume (Vcal) and recording the corresponding change in pressure (Pcal); K = Vcal/Pcal. For the recording of the breathing pattern, the flow through the chamber was momentarily interrupted by solenoid valves for a duration of ~2 min. This period of occlusion had negligible effects on the composition of the gases.

Breathing frequency (f, breaths/min) was computed from the breath-by-breath total cycle duration of the P recording, and tidal volume (VT, µl) was calculated from the P amplitude, K, Th, and the T and water vapor pressure of the respirometer (8). Pulmonary ventilation (Ve, ml/min) equals f × VT.

**Gaseous metabolism.** Immediately before sealing the chamber for the measurements of Ve, metabolic rate was measured by indirect calorimetry (oxygen consumption, VO2, and carbon dioxide production, VCO2) with an open-flow methodology (10) adapted to the avian embryo and hatching (21). A steady gas flow of 150 ml/min was continuously delivered through the respirometer, and the inflow and outflow O2 and CO2 concentrations were monitored by calibrated gas analyzers (FOX; Sable Systems International) after the gas had passed through a drying column. The output of the analyzers was displayed during online acquisition. VO2 and VCO2 were computed from the flow rate and the inflow-outflow concentration difference. The values (at standard T, pressure, and dry conditions) are presented in milliliters per minute.

**Protocols.** Once the hatching was placed in the respirometer, the inspired T of ~37.5°C, 30 min were given for acclimation. Then the gas inspired was switched to hypoxia (15% O2 and 10% O2 in that order, 20 min each) or to hypercapnia (2% CO2 and 4% CO2 in that order, 20 min each), with a period of air (30 min) in between the two exposures. The exposures to hypoxia and hypercapnia were in alternating order among animals, and recordings refer to the last 2–3 min of each exposure. Finally, after a new period in air (30 min), the animal was exposed to hyperoxia (85% O2) for 5 min, and the data refer to the last 2 min of this exposure period. Nx and Hx hatchlings were studied either during the early posthatching hours (on average 2- and 3-h-old for Nx and Hx, respectively) or later within the day (on average 18.5- and 20-h-old, respectively); none of these animals was studied at both times.

A second protocol was applied to a separate group of Nx and Hx hatchlings (n = 9 per group). In these animals only the hypoxic response was tested (30 min in air and 20 min in 10% O2), twice on the same animals, in the early (2.1 ± 0.4 and 2.3 ± 0.4 h for Nx and Hx, respectively) and late hours (20.3 ± 0.7 and 18.6 ± 1.2 h, respectively) of the first posthatching day. This was done to test longitudinally the results emerging from the main protocol (see RESULTS).

**Number of animals, data normalization, and statistics.** The numbers of animals used in the experiments are given in Tables 1 and 2. All group data are presented as means ± SE. Statistically significant differences between the Hx and Nx hatchlings were evaluated by two-tailed t-test. Statistical comparisons between the Hx andNx groups and between postnatal ages were done by two-way ANOVA, the first grouping factor being age, either unpaired (first protocol) or paired (second protocol), the second being the normoxic or hypoxic incubation, with post hoc Bonferroni’s limitations for the four comparisons of interest. In all cases, a difference was considered statistically significant at P < 0.05.

**RESULTS**

**Hatchlings.** A first analysis of the data was done by comparing all the hatchlings of the Nx (n = 33) and Hx groups (n = 30). They were all aged between 2 and 23 h after hatching, on average 6.8 ± 1.8- and 10.8 ± 1.8-h-old for Nx and Hx, respectively. These ages were not statistically different, nor were the starting egg weights and body weights at the time of the measurements. The only difference was in the incubation time; in fact, the Hx embryos, on average, hatched ~11 h later than the Nx embryos (Table 1).

In resting conditions, Ve in Hx was slightly higher than in Nx, with no differences in metabolic rate. Hence Ve/VO2...
Table 1. Average values of chicken hatchlings incubated in normoxia and hypoxia

<table>
<thead>
<tr>
<th></th>
<th>Normoxic Incubation</th>
<th>Hypoxic Incubation</th>
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</thead>
<tbody>
<tr>
<td>Number of animals</td>
<td>33</td>
<td>30</td>
</tr>
<tr>
<td>Egg mass at start, g</td>
<td>58.1±0.7</td>
<td>57.3±0.9</td>
</tr>
<tr>
<td>Weight at hatching, g</td>
<td>40.4±0.6</td>
<td>40.3±0.7</td>
</tr>
<tr>
<td>Incubation day at hatching</td>
<td>20.7±0.1</td>
<td>21.2±0.1</td>
</tr>
<tr>
<td>Age at experiment, h</td>
<td>6.8±1.8</td>
<td>10.8±1.8</td>
</tr>
<tr>
<td>Body temperature, °C</td>
<td>38.9±0.3</td>
<td>39.0±0.3</td>
</tr>
<tr>
<td>Tidal volume, μl</td>
<td>246.0±14.9</td>
<td>266.2±13.1</td>
</tr>
<tr>
<td>Breathing rate, breaths/min</td>
<td>70.8±2.9</td>
<td>74.3±2.6</td>
</tr>
<tr>
<td>Ve, ml/min</td>
<td>16.4±0.8</td>
<td>19.1±0.88</td>
</tr>
<tr>
<td>Vco2, ml/min</td>
<td>0.76±0.02</td>
<td>0.79±0.02</td>
</tr>
<tr>
<td>Vco2/Vco2</td>
<td>0.58±0.02</td>
<td>0.60±0.02</td>
</tr>
<tr>
<td>Vco2/Vco2O2</td>
<td>0.76±0.01</td>
<td>0.76±0.01</td>
</tr>
<tr>
<td>V/R Bangladesh STPD</td>
<td>21.8±0.9</td>
<td>24.7±1.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. All measurements were performed at the ambient temperature of 37.5°C; Ve, pulmonary ventilation; Vco2, oxygen consumption. Vco2, carbon dioxide production. *Statistically significant difference between groups (2-tailed t-test; P < 0.05).

1 The term “hyperventilation” is used to indicate an increase in Ve relative to metabolic needs, as expressed by an increase in Ve/Vco2. The term “hypoxia” is used to indicate an increase in the absolute value of Ve.

The main finding of this study was that prenatal hypoxia caused a decrease in the ventilatory chemosensitivity of the 1-day-old hatching, with ventilatory chemosensitivity defined as the relative increase in Ve/Vco2 from the air value. This drop was also apparent after separating the results into early and late hours, to take into account the changes in chemosensitivity occurring within the first day, and the changes in the control of
heat production (1). The lower hypoxic sensitivity of Hx was confirmed further by repeated measurements within the same Hx hatchlings at the early and late hours. Before commenting on these results, it seems appropriate to discuss briefly some aspects of the methodology and of the VE responses of the normoxic group, given the paucity of information on resting breathing and ventilatory chemosensitivity in neonatal birds.

Methods and protocols. The barometric technique has the advantage of noninvasiveness, but it is prone to errors in the computation of VT when the T difference between body and ambient is small (26). The present setup, therefore, was designed to retain a rather large T difference (almost 10°C) while keeping the hatchling at its customary ambient T of ~38°C (40). Because these conditions remained the same for both experimental groups and responses were analyzed as percent of the air values, it is extremely unlikely that the observed differences in chemosensitivity between Nx and Hx reflected a systematic error introduced by the methodology. Furthermore, some of the differences in hyperventilation between the two groups were due to differences in f and in VO2 (Fig. 2), neither of which is influenced by the barometric methodology.

The complete experiment, with exposure to two levels of hypoxia and hypercapnia and with a period of air breathing in between, lasted >2 h. Hence the main protocol was based on a cross-sectional design, where each hatchling was studied once, either in the early or late hours of the first posthatching day. This eliminated any possibility of carry-over effects from the earlier exposure but left open the possibility that unaccounted differences between the groups may have contributed to the results. Therefore, we have chosen to extend the measurements to another group of hatchlings studied longitudinally during the early and late hours of their first postnatal day. This second protocol was of shorter duration (~45 min), because it included only the exposure to 10% O2, chosen because, with
the former protocol, this exposure had caused the largest difference in hyperventilation between Nx and Hx. The results of this second protocol confirmed those of the main protocol.

**Hypoxic and hypercapnic hyperventilation.** Both the arterial O$_2$-sensing and the intrapulmonary CO$_2$-sensitive chemoreceptors are functional during the paranatal phases of the hatching process, although demonstrations by neural recordings have been obtained only for the latter receptors (34). Data in the shearwater (33) and in the chicken (22) have shown that the V˙E responses to hypoxia and hypercapnia are already manifest in embryos during the early hatching phases, becoming stronger after hatching, prevalently through the increase in VT. The current measurements indicated that the progression in the V˙E chemosensitivity (evaluated as hyperpnea or hyperventilation) continued within the first day, increasing from the early to the late hours, and that VT was the major contributor to the hyperpnea both in hypoxia and in hypercapnia.

In resting conditions, the V˙O$_2$ of the hatchlings (0.76 ml/min or ~19 ml·kg$^{-1}$·min$^{-1}$) corresponded to what should be expected at 38°C (1, 25, 28). The V˙E/V˙O$_2$ of ~22 was low compared with newborn or adult mammals of similar body size (24) but was quite similar to the V˙E/V˙O$_2$ values of 21–22 of adult birds (2, 43), including the domestic fowl (6). The lower V˙E/V˙O$_2$ of birds compared with mammals reflects their better gas-exchange efficiency (5, 38), a characteristic that, therefore, is already apparent at birth.

The mechanisms behind the hypoxic decrease in metabolic rate, responsible for more than half of the hypoxic hyperventilation and quite common in newborn mammals (24), are unclear, although it is known that they do not involve recruitment of anaerobic energy sources (25). The fact that the drop in V˙O$_2$ did not happen with hypercapnia demonstrates that the hypometabolic response is hypoxia specific and not a generalized response to chemoreceptor stimulation. It is interesting

![Fig. 3. Changes in ventilatory parameters and in V˙O$_2$ during hypercapnia, expressed as percent of air value (dashed line), in hatchlings during first posthatching day. Symbols are means; bars indicate SE; n = 30 for both groups. *Statistically significant difference between groups.](image-url)

![Fig. 4. Average values of body temperature in hatchlings during air breathing in hypoxia (left) or hypercapnia (right). Symbols are means; bars indicate SE. Numbers refer to number of animals studied. *Statistically significant difference between groups.](image-url)
that Tb decreased both in hypoxia and hypercapnia, although hypometabolism occurred only in the former case. Hence, whereas in hypoxia the hypometabolism was the main cause of the drop in Tb, in hypercapnia Tb decreased because of the increase in heat loss, to which the hyperpnea itself must have been a major contributor.

Finally, it is worth noting the absence of \( f \) increase in response to hypoxia (Fig. 2). Also, embryos during the hatching phases show only a modest or no increase in \( f \) during acute hypoxia or anoxia (22, 33), in contrast to the response of adult birds (38). The small (and not significant) drop in \( V_E \) during hyperoxia (Fig. 7) suggests that in these first postnatal hours

### Table 2. Average values of chicken hatchlings incubated in normoxia and hypoxia, measured during early or late hours of first posthatching day

<table>
<thead>
<tr>
<th></th>
<th>Normoxic Incubation</th>
<th>Hypoxic Incubation</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Early</td>
<td>Late</td>
</tr>
<tr>
<td>Number of animals</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>Egg mass at start, g</td>
<td>56.8±1.0</td>
<td>59.3±1.0</td>
</tr>
<tr>
<td>Weight at hatching, g</td>
<td>41.1±0.8</td>
<td>39.9±0.9</td>
</tr>
<tr>
<td>Incubation day at hatching</td>
<td>20.3±0.5</td>
<td>20.8±0.1a</td>
</tr>
<tr>
<td>Age at experiment, h</td>
<td>2.3±0.5</td>
<td>18.5±1.9a</td>
</tr>
<tr>
<td>Body temperature, °C</td>
<td>37.9±0.3</td>
<td>39.9±0.2</td>
</tr>
<tr>
<td>Tidal volume, µl</td>
<td>209.5±21.2</td>
<td>276.3±18.4a</td>
</tr>
<tr>
<td>Breathing rate, breaths/min</td>
<td>81.7±3.8</td>
<td>61.7±2.8a</td>
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<tr>
<td>( V_E ), ml/min</td>
<td>16.2±1.2</td>
<td>16.6±1.2</td>
</tr>
<tr>
<td>( V_O_2 ), ml/min</td>
<td>0.78±0.02</td>
<td>0.74±0.03</td>
</tr>
<tr>
<td>( V_C O_2 ), ml/min</td>
<td>0.61±0.02</td>
<td>0.55±0.02</td>
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<tr>
<td>( V_C O_2/V_O_2 )</td>
<td>0.78±0.01</td>
<td>0.75±0.01a</td>
</tr>
<tr>
<td>( V_E/V_O_2 ) BTPS/STPD</td>
<td>20.9±1.4</td>
<td>22.5±1.2</td>
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</table>

Values are means ± 1 ± SE. All measurements were performed at the ambient temperature of 37.5°C. *Statistically significant difference between early and late; **statistically significant difference between groups of same age (two-way ANOVA with 2 grouping factors; \( P < 0.05 \)).

Fig. 5. Changes in \( V_E, V_O_2, \) and \( V_E/V_O_2 \) during hypoxia, expressed as percent of air value (dashed line), in two different groups of hatchlings (H1) studied either during early (left) or late (right) of first posthatching day. Symbols are means; bars indicate SE; \( n = 15 \) for both groups. *Statistically significant difference from corresponding value at younger age; **statistically significant difference between groups (2-way ANOVA with post hoc Bonferroni’s limitations for 4 comparisons of interest, 1st factor being postnatal hours, 2nd factor being treatment during incubation).
the hypoxic tonic drive to $V_E$ is minimal. This differs from what has been noticed in adult birds (38) but is similar to the situation in neonatal mammals (24).

**Hx group: resting conditions.** Embryonic hypoxia is known to decrease body growth; however, the additional 10–12 h of incubation allow the Hx embryos to incorporate the remaining yolk and reach a body weight similar to that of Nx (1). At that time, in resting conditions, there were virtually no differences in metabolic and breathing parameters between Hx and Nx hatchlings, except for a slightly higher $V_E$ (and $V_E/V_O_2$) in Hx (Table 1). This modest resting hyperventilation is quite similar to that observed in rat pups born after hypoxic gestation (12). In that case, hyperoxia did not reduce the hyperventilation, which, therefore, was attributed to hypoxemia caused by the postnatal persistence of fetal right-to-left vascular shunts. It is possible that the same may apply to the Hx hatchlings, although during hyperoxia the small differences in $V_E$ from the Nx group did not reach statistical significance.

**Hx group: ventilatory chemosensitivity.** Many steps are involved in the reflex arch of $V_E$ chemosensitivity, and hypoxia-induced plasticity could occur at any level, including signal transduction, central integration of peripheral inputs, output generation, and translation into mechanical events. In newborn and adult mammals, there is evidence of plasticity of the hypoxic ventilatory response mainly at the level of the carotid body (7, 18, 35), although one study suggests that plasticity may occur at the neuromechanical level of the reflex loop (3). The blunting of the hypoxic and hypercapnic hyperventilation in the hatchlings after embryonic hypoxia is reminiscent of what has been observed in adult mammals after a sustained alteration of the inspired $O_2$ level during the early postnatal period. In fact, adult rats maintained in hyperoxia for 4 wk from birth presented a blunted $V_E$ response to acute hypoxia; the most likely factor responsible for their diminished hypoxic response was identified in an impairment of the transduction properties of the peripheral chemoreceptors (18).
Similarly to hyperoxia, hypoxia, either intermittent or sustained, during the early postnatal weeks also modified the respiratory regulation of the adult, with a blunted hypoxic $\dot{V}E$ response (30, 31, 36). The importance of having a correct level of oxygenation for the normal development of the carotid body during the postnatal period could also apply during the embryonic phases. In the chicken, the early buds of the carotid bodies reach their final location at the bifurcation of the brachycephalic arterial trunk by embryonic day 8 (29), and the characteristics of mature glomus cells are recognized a few days later (15). Hence, if the blunting in hypoxic hyperventilation were due mainly to the effects of hypoxia on the functional development of the carotid body, it would mean that this occurred during the last half of the embryonic period. However, the decreased chemosensitivity found in the Hx hatchlings was not limited to the hypoxic ventilatory response; it also included the metabolic adaptation to hypoxia, which should not depend on the carotid bodies (27). Hence the effects of embryonic hypoxia on the ventilatory chemosensitivity of the hatchling must have been the result of a broad spectrum of mechanisms and were not limited to the carotid bodies.

The intrapulmonary chemoreceptors, from the viewpoint of the regulation of the breathing pattern, are the avian equivalent of the mammalian slowly adapting pulmonary stretch receptors (38). They are functional during the paranatal phases of the hatching process (34), but whether or not their firing characteristics or the central integration of their inputs are influenced by hypoxia during embryonic development is not known.

Although there were some differences between the early and late hours, and between the results of the first (transversal) and second (longitudinal) protocol, hypometabolism during hypoxia occurred to a lesser degree in Hx than in Nx (Fig. 3). In parallel with this was the finding that, in hypoxia, Tb decreased less in Hx than in Nx (Fig. 2). Presumably, the reluctance of Hx to drop VO$_2$ in hypoxia reflected the need to pay back some lactic acid accumulation and O$_2$ debt contracted during the hatching effort or the compensatory catch-up growth on return to normoxia. In fact, a small tendency to maintain a higher resting VO$_2$ was detectable also in normoxia, and it was observed in previous experiments on 1-day-old hatchlings after hypoxic incubation (1). Also, the hypoxia-induced catecholamine release could help the Hx hatchlings to maintain VO$_2$ in hypoxia (45). Alternatively, if, as mentioned earlier, the hatchlings of the Hx group were hypoxemic, the higher VO$_2$ could have been a measure to protect their Tb and facilitate the delivery of O$_2$ to the peripheral tissues.

The current data cannot totally exclude the possibility that a mechanical impairment of the respiratory apparatus (drop in compliance or increased resistance) might have contributed to the decreased $\dot{V}E$ responses in Hx, but this eventuality seems quite remote. In fact, the $\dot{V}E$ levels reached by the Hx hatchlings during 2 and 4% CO$_2$ breathing (140 and 160% of resting $\dot{V}E$, respectively) were far above the highest values reached in hypoxia (108%) (Figs. 2 and 3). This implies that, from the viewpoint of respiratory mechanics, the Hx animals had the possibility of reaching values of $\dot{V}E$ similar to those of the Nx hatchlings.
group. There are no indications that embryonic hypoxia hinders the surfactant system; on the contrary, in the chicken embryo hypoxia accelerates the maturation of surfactant, possibly through increased corticosterone production (4).

Conclusions. In the chicken, sustained hypoxia during the embryonic development leads to a blunted Ve chemosensitivity in the newborn. The mechanisms for this effect are not clear, nor is it possible to anticipate when and to what extent chemosensitivity may eventually recover during postnatal life. The fact that embryonic hypoxia can have a profound influence on the development of the regulation of breathing has biological implications, because it represents an important example of an environmental situation that can influence the phenotypic expression of a genetic trait. In addition, it could carry clinical significance, also considering that the malfunction of the peripheral chemoreceptors is considered a likely culprit in the pathogenesis of sudden infant death syndrome (11). Indeed, if extrapolated to humans, the data suggest that fetal hypoxia, as it occurs with maternal smoking or at high altitude, can hinder the capacity of the newborn baby to cope with hypoxic episodes, which can be frequent events in the perinatal period.

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REFERENCES


