THE ACID-BASE STATUS OF THE BLOOD OF THE DEVELOPING CHICK EMBRYO

BY C. DAWES AND K. SIMKISS

Department of Physiology and Biochemistry, The University, Reading, Berks.

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INTRODUCTION

The avian embryo develops within a cleidoic egg which largely insulates it from the environment. There is an exchange of respiratory gases into the air space of the egg and into the surrounding air via the pores in all parts of the eggshell, but as development progresses and the metabolism of the embryo increases this exchange appears to become incomplete. Thus, in the egg of the domestic fowl, Visschedijk (1962) found that the carbon dioxide in the air space increased up to 5.6% during the time when the chorio-allantoic membrane was acting as a respiratory surface and rose further to 6.6% during the period of pulmonary respiration before hatching. Shortly before the shell was pipped there was an average carbon dioxide concentration of about 8% in the air space. The carbon dioxide tension of the blood of avian embryos has never been measured, but it appears likely, from the air space analyses, that the embryo would be in a state of progressive respiratory acidosis during the latter half of its development. This possibility would be in agreement with the observations of Beattie (1964), who found evidence for a rapid release of carbon dioxide from chick embryos at the time of hatching.

A number of attempts have been made to measure the pH of chick embryonic blood. According to Cohn & Mirsky (1929) oxygenated blood from 8-day embryos had a pH of 7.03 which rose to 7.23 on the 20th day of incubation. Their data are, however, extremely variable and they had to dilute the blood with saline in order to have a sufficiently large sample to analyse. More recently Abramovici (1967) has used microelectrodes to follow the changes in pH of the body fluids of chick embryos. He obtained values of 7.4–7.5 on days 9–10 and a steady pH of 7.64 ± 0.05 for the remaining period of incubation. These values were obtained from plasma rather than whole blood so that there is the possibility of some slight change in pH during the preparation of the specimens.

The sparse evidence available suggests, therefore, that although there may be a rise in carbon dioxide tension the pH of the blood does not fall during development. In most vertebrates this would be interpreted as a renal compensation for respiratory acidosis and would be accompanied by an excretion of hydrogen ions in the urine (Pitts, 1963). It has, in fact, been shown on a number of occasions that the allantoic fluid of the avian embryo changes from a slightly alkaline pH of over 7.4 during the first 2 weeks of incubation to a pH of 5.8 on the 14–15th day of incubation (Shkylar, * Present address, Department of Zoology, Queen Mary College, London, E. 1.

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The understanding of these acid-base disturbances is complicated by two facts. First, there are many developmental phenomena which complicate any simple analysis of the acid-base changes. Thus, there is a doubling of the 'total solids' content of the blood between day 10 and the end of incubation (Schechtman, 1952), and the main buffer of the blood, haemoglobin, also increases. On day 9 the haemoglobin content is 4.8 g.%, rising steadily to 5.5 g.% (day 12), 8.2 g.% (day 15) and 10.1 g.% (day 18) (Barnes & Jensen, 1959). The composition of the haemoglobin also changes (Simons, 1966). Superimposed upon these changes in blood buffers is a second complicating factor, namely that the embryo resorbs the inner parts of the calcareous eggshell during the latter part of incubation. About 100 mg. of calcium are obtained and used by the embryo for bone formation (Simkiss, 1967) but nothing is known about the use of the associated carbonate. It has been assumed (Romanoff, 1967) that it is converted into carbon dioxide but this greatly oversimplifies the situation and ignores the fact that it is an important buffer for the developing embryo.

Almost none of these intriguing changes in the chemistry of the avian embryo have been related to the way in which the developing bird defends itself against the various acidotic and alkalotic influences to which it is normally exposed. The following investigation was therefore undertaken in order to determine the pH, $P_{CO_2}$, and bicarbonate levels of embryonic blood at various stages of incubation. These analyses provide a basis for delimiting the extent of the problem, and by combining them with estimates of 'base excess' it is also possible to relate any disturbance and its compensation to respiratory or non-respiratory influences.

**MATERIALS AND METHODS**

Fertile eggs of the domestic fowl (White Leghorns) were used throughout this work. They were incubated at 103°F. in commercial 'still-air' incubators using normal practice. The degree of development of the embryos was determined firstly by timing their period of incubation and secondly by measuring their 3rd toe length and relating this to the developmental stages of Hamburger & Hamilton (1951). The results are expressed as 'days of incubation', but it should be realized that this is only partly a chronological scale and also involves reference to a scale of normal development.

Blood was collected anaerobically from the embryos by opening the shell and inserting a heparinized capillary tube into the heart. It is not possible to collect sufficient blood for analysis before the 11th day of incubation so that the experimental observations are restricted to the latter half of the incubation period. The cardiac puncture technique is a satisfactory way of collecting blood up until the last few days of incubation when the animals are inclined to struggle. This was avoided in hatchlings by immediately decapitating the animals and collecting fresh blood from the bleeding surfaces into the heparinized capillary tubes.

Three samples, each containing about 70 μl. of blood, were normally collected from each embryo and analysed by the methods of Sigaard-Andersen (1965). Two samples were equilibrated in an Astrup microtonometer with 4% and 8% CO$_2$/O$_2$ gas mixtures whose composition had previously been accurately determined by
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volumetric analysis. The pH of the equilibrated blood was determined with a Radiomter microelectrode maintained at 38° C. The pH of normal blood was also measured with the same instrument. The log. $P_{CO_2}/pH$ relationship of blood is normally linear and this was confirmed for embryonic blood. It is therefore possible to plot the pH and log. $P_{CO_2}$ values for the equilibrated samples and thus to determine graphically $P_{CO_2}$ of the normal blood from its pH value. The slope of the log $P_{CO_2}/pH$ line is a measure of the buffering ability of the blood and the position of the line is a measure of the base excess. This is defined as ‘the titratable base on titration to normal pH at normal $P_{CO_2}$ and normal temperature’ (Sigaard-Andersen, 1965).

Base excess and bicarbonate values can be determined from the log $P_{CO_2}/pH$ lines using nomograms prepared for human blood. Alternatively it is possible to calculate the bicarbonate values of embryonic blood by applying the Henderson–Hasselbalch equation

$$pH = pK_1 + \log \frac{HCO_3}{\alpha CO_2},$$

using a value of 6.09 for $pK_1$ (Helbacka, Casterline, Smith and Shaffner, 1964) and 0.032 for $\alpha$ (Severinghaus, Stupfel & Bradley, 1956).

In the above experiments values of $P_{CO_2}$ were obtained by graphical analysis. The carbon dioxide tension has also been determined directly, however, by using a Severinghaus microelectrode. Although this electrode only requires about 70 μl. blood it is often not possible to obtain this extra sample when all the above analyses are also performed. The experiment was therefore performed in a separate set of analyses (series B, Table 1) on the 13th, 15th and 18th days of incubation and on 2-day-old hatchlings.

RESULTS

The changes in pH, $P_{CO_2}$, bicarbonate and base excess levels of embryonic blood are shown in Fig. 1 for the series A experiments. The actual values (means ± s.d.) are shown in Table 1. The day-to-day variations in the values are not significantly different, as assessed by Students t test, except for the changes in bicarbonate and base excess levels during days 13–16 ($P < 0.001$) and for the fall in pH on day 19 ($P < 0.01$).

Continuous sampling of the blood from individual embryos showed very little variation in pH with time, providing the blood flowed easily during the cardiac puncture. The variations in $P_{CO_2}$ were checked by comparing the values of carbon dioxide determined graphically from the nomograms of Sigaard-Anderson (series A) with actual determinations using the carbon dioxide electrode (series B). The results are shown in Table 1. The two sets of readings were compared using Students t test and showed no significant difference although the values obtained from the carbon dioxide electrode were generally slightly higher than the readings obtained graphically.

The values of $P_{CO_2}$ plotted in Fig. 1 show that there is a continuous rise from 11 to 19 days with the greatest rate of increase being in the 12- to 14-day period. After 19 days the carbon dioxide tension of the blood falls quickly until hatching.

The increase in blood bicarbonate (Table 1) is due to two effects. First, there is a direct increase due to the increase in carbon dioxide tension, and secondly, there is an increase due to the addition of extra base to the blood. This second effect is by far the most important in the present work and can be isolated from the effects of
Table 1. Changes in pH, $P_{CO_3}$, bicarbonate and base excess levels of the blood of chick embryos during development

(Figures refer to mean values ± S.D. The number of observations involved are shown in parentheses after each mean.)

<table>
<thead>
<tr>
<th>Embryo age</th>
<th>pH</th>
<th>$P_{CO_3}$ (mm Hg)</th>
<th>Bicarbonate (m-equiv/l.)</th>
<th>Base excess (m-equiv/l.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Series A</td>
<td>Series B</td>
<td>Series A</td>
<td>Series B</td>
</tr>
<tr>
<td>11 13-14</td>
<td>7.475 ± 0.055 (8)</td>
<td>7.425 ± 0.022 (10)</td>
<td>7.387 ± 0.030 (12)</td>
<td>7.425 ± 0.013† (12)</td>
</tr>
<tr>
<td>12 13-14</td>
<td>7.365 ± 0.036 (16)</td>
<td>7.388 ± 0.041 (16)</td>
<td>7.406 ± 0.030 (10)</td>
<td>7.371 ± 0.035* (8)</td>
</tr>
<tr>
<td>13 14-15</td>
<td>7.430 ± 0.025 (13)</td>
<td>7.411 ± 0.034 (11)</td>
<td>7.412 ± 0.044 (10)</td>
<td>7.370 ± 0.031† (9)</td>
</tr>
<tr>
<td>14 15-16</td>
<td>7.334 ± 0.014 (12)</td>
<td>7.418 ± 0.009 (11)</td>
<td>7.458 ± 0.017 (3)</td>
<td>7.418 ± 0.037 (6)</td>
</tr>
<tr>
<td>15 16-17</td>
<td>7.427 ± 0.040 (10)</td>
<td>7.423 ± 0.037 (6)</td>
<td>7.415 ± 0.011† (7)</td>
<td>22.3 ± 0.2 (7)</td>
</tr>
</tbody>
</table>

Statistical significance between series A and B assessed by Students $t$ test.
† Not significant; * $P < 0.05$; ** $P < 0.01$.
$P_{CO_3}$ values of series A determined graphically.
$P_{CO_3}$ values of series B determined with a carbon dioxide electrode.
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carbon dioxide variations by correcting all values to a standard $P_{\text{CO}_2}$ of 40 mm. Hg. These corrected values are shown as 'base excess' levels in Fig. 1.

The pH of the blood remains throughout the incubation period within narrow limits. The mean value of 129 readings taken between the 11th and 21st days of incubation was $7.401 \pm 0.050$. There are, however, two tendencies for it to fall, reaching $7.365 \pm 0.036$ on days 13-14 and $7.334 \pm 0.040$ on day 19 (Fig. 1). These values were highly significantly different ($P < 0.001$) from the mean values of all readings as assessed by Students $t$ test.

![Figure 1: Changes in blood pH, base excess, and carbon dioxide tension during development of chick embryos.](image)

Fig. 1. The changes in blood pH, base excess, and carbon dioxide tension during the development of chick embryos. The time of hatching is arbitrarily fixed at 21 days and the data for 1- and 2-day-old hatchlings are also included. The circles represent mean values, the standard deviations of which are shown in Table 1, series A.
DISCUSSION

It is apparent that the following are the main acid-base changes in the blood during incubation.

1. A continuous rise in $P_{CO_2}$ from a value of about 19 mm. Hg on day 11 to about 57 mm. Hg on day 19.

2. A rapid rise in bicarbonate content which occurs mainly during the period from 12 to 16 days when the level changes from about 16 to 33 m-equiv./l.

3. A similar rapid change in base excess from $-8$ to $+6.8$ m-equiv./l. during the same 4-day period.

It is thus apparent that the large change in blood bicarbonate (17 m-equiv./l. in 4 days) is mainly due to a change in base excess (15 m-equiv./l. in 4 days) which indicates an influx of extra bicarbonate into the blood. This influx of base lags slightly behind the rise in $P_{CO_2}$ by about 24 hr., although it should be realized that this analysis is based entirely upon averaged data. The ratio of $HCO_3^/-aCO_2$ passes from a value of 24.2 on day 11 to a minimum of 19.0 in the period 13–14 days, before rising again to a value of 23.4 on day 17. The ratio of the buffer components $HCO_3^-/H_2CO_3$ determines the pH of the blood so that the fall in pH on day 13–14 can be interpreted as due to the lag in the increase of bicarbonate (Fig. 1).

The level of base excess in the blood reaches a plateau between days 16 and 20, although the $P_{CO_2}$ continues to rise until day 19. Again therefore the ratio $HCO_3^-/aCO_2$ falls to 17.7, so that there is a corresponding minimum pH value on day 19 (Fig. 1). After this time the $P_{CO_2}$ starts to fall as the embryo assumes pulmonary respiration and pips the shell. Thus, for a short time the ratio $HCO_3^-/aCO_2$ rises and the embryo is in a temporary state of alkalosis. This soon passes, however, as the bicarbonate level of the blood decreases from about 34 m-equiv./l. on day 20 to 25 m-equiv./l. on the day after hatching.

The changes in blood pH can therefore largely be explained on the basis of two variables, i.e. the carbon dioxide tension and the blood bicarbonate levels.

The continuous rise in $P_{CO_2}$ until day 19 is explicable on the basis of the increasing metabolism of the embryo. The fall in the last 2 days of incubation is due to the fact that the analyses include values for embryos which had pipped the shell and thus had easier access to the surrounding air. It is interesting to note, however, that according to Visschedijk (1962) the carbon dioxide concentration in the air space increases up to 5.6% during chlorio-alantoic respiration and then up to 6.6% when pulmonary respiration starts. The value of 6.6% carbon dioxide is similar to the value of 50 mm. Hg found in the blood of 20-day embryos in the present study. This, however, is less than occurs in the blood during earlier stages of incubation, which indicates either that the two studies are not directly comparable or that the composition of the air space does not accurately reflect the carbon dioxide tension of the blood until pulmonary respiration starts (i.e. around day 20). This assumes, of course, that the blood samples obtained from the embryos represent the composition of the blood in vivo and are not modified by the struggling of the embryo during sampling. These assumptions appear to be largely justified since successive samples show little change in pH and the differences found in the last 2 days of incubation, when struggling is most violent, are in the opposite direction to what might have
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been expected with increased muscular activity, i.e. the blood pH rises and $P_{CO_2}$ falls.

The changes in blood bicarbonate levels are largely due to an influx of extra 'non-respiratory' bicarbonate as the base excess curves indicate. The increase occurs in the period from day 12 to day 16 and is most likely to come from either the resorption of the eggshell, the activity of the kidney, or both. The chorio-allantoic membrane first touches the eggshell on about day 10 of incubation but does not reach its maximum size until about day 14. Throughout the remainder of the incubation period the chorio-allantois lines the inner part of the calcareous eggshell and resorbs mineral from it. About 100 mg. calcium enter the chick embryo by this route (Johnston & Comar, 1955), so that about 150 mg. of carbonate ions would also be made available to the blood. If it is assumed that this enters the embryo and is spread evenly throughout 20 g. of tissue this would raise the bicarbonate concentration by 250 m-equiv./l. The pH in such a case can be calculated from the Henderson–Hasselbalch equation on the basis of a maximum $P_{CO_2}$ of 60 mm. Hg (Table 1), i.e.

$$
\text{pH} = 6.1 + \log \frac{250}{0.03 \times 60} = 8.2.
$$

It is apparent from the pH measurements shown in Table 1 that this value is never attained. Thus, the carbonate of the shell does not remain in solution within the tissues and the embryo must, in some way, control its bicarbonate content. The limits of normal variation found in this study are from 15 m-equiv./l. on day 11 to a maximum bicarbonate concentration of about 35 m-equiv./l. on day 18 (Fig. 1). In this respect it is interesting to note that Harsch & Green (1963) found a corresponding change in the electrolyte content of plasma between the 11th and 18th days of incubation. Their analyses showed that the sodium and potassium content rose from 145 to 154 m-equiv./l., while the chloride concentration fell from 110 to 98 m-equiv./l. during this period. Thus, as they pointed out, there must be a corresponding increase of about 21 m-equiv./l. in some unknown anion. The rise of 20 m-equiv./l. in blood bicarbonate levels found in the present study would thus account for this anion deficiency and so maintain the electro-neutrality of the blood.

The other organ most likely to influence the bicarbonate levels of the blood is the kidney. This contains the enzyme carbonic anhydrase from about day 10 onwards (Clark, 1951) and this, together with the acidification of the allantoic fluid after day 15 (Abramovici, 1967) could be correlated with at least part of the increase in base excess. Unfortunately, relatively little is known about this aspect of developmental physiology. It is apparent, however, that a number of organs and a number of sources could be invoked to account for the observed changes in blood bicarbonate. It may be better therefore to approach the problem by attempting to understand how the bicarbonate levels are controlled in order to maintain the remarkably stable blood pH which is found during development, and some form of metabolic acidosis may have to be invoked here in order to explain how the embryo controls the enormous source of base available from the resorbed eggshell.
SUMMARY

1. The pH, carbon dioxide tension, bicarbonate and base excess levels of chick embryos have been measured during the period of 11 days of incubation until the 2nd day post hatching.

2. The carbon dioxide tension rises continuously from a value of about 20 mm. Hg on day 11 to a maximum of almost 60 mm. Hg on day 19.

3. The bicarbonate content rises rapidly from the 12th day (16 m-equiv./l.) until the 16th day (33 m-equiv./l.).

4. The pH falls to minimum values on the 13–14th day and the 19th day.

5. These variations are discussed in relation to the physiology of the developing embryo and its acid-base metabolism.

REFERENCES


