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# THE EFFECTS OF RESPIRATORY ACIDOSIS IN THE CHICK EMBRYO

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## INTRODUCTION

The avian embryo develops within an eggshell which limits the rate of gaseous exhange between the bloodstream and the environment. In the latter half of normal incubation a considerable respiratory disturbance develops so that in the blood of the embryonic fowl the $P_{CO_2}$ rises from 20 to 60 mmHg within about 8 days (Dawes & Simkiss, 1969; Freeman & Misson, 1970). A variation of this magnitude in the adult fowl would cause a change in blood pH from 7·7 to 7·2 in the absence of any compensatory adjustments. In fact the adult fowl appears to be relatively poor in compensating for respiratory acidosis (K. Simkiss, unpublished), and in calculating the figures shown in Table 1 it has been assumed that there is virtually no change in plasma bicarbonate during an acute respiratory acidosis (Hunt & Simkiss, 1967). In contrast to this it has been shown that in the embryo there is a large increase in plasma bicarbonate during normal development so that the change in blood pH is greatly reduced (Dawes & Simkiss, 1969). The situation is shown in Table 1 with the implication that the pH of the blood is less variable in the embryo than it would be in the adult fowl subjected to similar circumstances.

Table 1. *Changes in blood pH and bicarbonate content in relation to the normal change in $P_{CO_2}$ of embryos and in adults exposed to acute changes in $P_{CO_2}$ of the same magnitude*

| | Embryo | | Adult | |
|---|---|---|---|---|
| | 11 days | 18 days | — | — |
| $P_{CO_2}$ (mmHg) | 20 | 60 | 20 | 60 |
| pH | 7·5 | 7·3 | 7·7 | 7·2 |
| $HCO_3^-$ (m-equiv./l) | 15 | 38 | 24 | 24 |

There appear to be two possible explanations for the rapid rise in plasma bicarbonate which occurs during the period of from 11 to 18 days of incubation and which results in the constancy of the pH of embryonic blood during the latter half of incubation. Either the avian embryo normally undergoes some developmental process at this time which affects the body buffers, or alternatively the embryo possesses some adaptive mechanism whereby it can rapidly compensate for an increase in carbon dioxide tension. If this is the case the animal presumably loses this ability in later life. The

following experiments were therefore undertaken in an attempt to obtain further information on the responses of the embryo of the fowl to an additional increase in $P_{CO_2}$ during development.

## MATERIALS AND METHODS

Fertile eggs of the domestic fowl (White Leghorn) were incubated at 39·4 °C (103 °F) in a commercial still-air incubator. After 9 days of normal incubation some of these eggs were removed to a small metal 'Brower' incubator with a capacity of 50 eggs. A mixture of 9% carbon dioxide in air was prepared with a Wosthoff gas-mixing pump and this was circulated through the incubator. The output of the incubator was continuously monitored by means of a Cambridge katharometer and recorder.

Blood samples were collected from experimental and control eggs during the period of 12–17 days incubation and analysed for pH, $P_{CO_2}$, bicarbonate and base excess using the methods of Siggaard-Anderson (1965) as previously described (Dawes & Simkiss, 1969). Allantoic fluid was removed by carefully puncturing the egg through the air space and allowing the fluid to drain into a funnel. Any solid deposits left in the allantois were later removed and added to the fluid samples. Care was taken throughout these operations to avoid contamination of samples with blood. The volume of fluid obtained was measured and its pH was recorded as soon as possible. Ammonia was determined in allantoic fluid by the microdiffusion method of Conway (1947). The titratable acidity of the allantoic contents was determined with a Radiometer autotitrator using either 0·1 N hydrochloric acid or 0·1 N sodium hydroxide. Considerable difficulty was encountered in obtaining accurate readings of titratable acidity since the early samples (12–14 days) are alkaline and lose carbon dioxide at variable rates during titration. Samples from 14 to 15 days onwards tend to be acid and contain deposits of uric acid. These act as strong buffers and are particularly difficult to titrate unless dissolved. Back titrations of both types of samples give poor recoveries. These problems are described in detail by Dawes (1970). In practice it was decided to titrate the samples until the deviation from pH 7·4 was less than 0·01 units in 5 min. The calcium content of the embryo and of the whole egg contents was determined by digesting the samples in nitric/perchloric acid mixtures, adding 2·5 g/l strontium chloride and analysing samples in a Hilger & Watts atomic absorption spectrophotometer.

In previous work it was noted that there was considerable variation in the degree of development of similarly 'aged' eggs (Dawes & Simkiss, 1969). This was corrected by measuring the third toe lengths which are a good indication of the stage of development (Hamburger & Hamilton, 1951). In the present work it was necessary to kill embryos at certain stages of development. This could only be planned on a chronological basis, but the toe lengths were measured at the end of the experiment so that reference could be made to earlier results.

## RESULTS

Changes in blood pH, $P_{CO_2}$ and bicarbonate levels for control and hypercapnic embryos are given in Table 2 for the period 12–17 days incubation. The values for carbon dioxide tension and base excess are given in Figs. 1 and 2. It was necessary to make some slight adjustments to the gas-mixing pump during the experiment so as to maintain the $P_{CO_2}$ at a fairly constant level despite increased endogenous carbon

Table 2. *Changes in blood pH, $P_{CO_2}$ and bicarbonate during incubation in air and in 9% carbon dioxide*

(Mean values ± S.D. Number of samples in parenthesis.)

| Days of incubation | | 12 day | 13 day | 14 day | 15 day | 16 day | 17 day |
|---|---|---|---|---|---|---|---|
| pH | Air | 7·370 ± 0·062 (6) | 7·387 ± 0·042 (6) | 7·353 ± 0·049 (6) | 7·360 ± 0·056 (6) | 7·414 ± 0·033 (7) | 7·405 ± 0·029 (8) |
| | 9% $CO_2$ | 7·231 ± 0·036 (6) | 7·265 ± 0·026 (6) | 7·333 ± 0·077 (6) | 7·304 ± 0·080 (6) | 7·296 ± 0·052 (6) | 7·293 ± 0·029 (4) |
| $P_{CO_2}$ (mmHg) | Air | 30·9 ± 11·9 (6) | 37·4 ± 7·1 (6) | 48·6 ± 11·8 (6) | 46·9 ± 5·2 (6) | 47·4 ± 10·8 (7) | 50·3 ± 6·4 (8) |
| | 9% $CO_2$ | 87·6 ± 22·0 (6) | 85·2 ± 15·6 (6) | 83·5 ± 15·4 (6) | 95·1 ± 23·8 (6) | 86·0 ± 15·2 (6) | 92·5 ± 5·2 (4) |
| $HCO_3^-$ (m-equiv./l) | Air | 18·60 ± 6·56 (6) | 23·69 ± 3·10 (6) | 28·24 ± 4·38 (6) | 28·49 ± 5·75 (6) | 32·00 ± 5·13 (7) | 33·32 ± 3·08 (8) |
| | 9% $CO_2$ | 38·87 ± 8·83 (6) | 40·93 ± 6·00 (6) | 46·71 ± 6·96 (6) | 49·46 ± 8·59 (6) | 44·17 ± 3·24 (6) | 47·56 ± 3·03 (4) |

Table 3. *Changes in pH, volume, and titratable acidity of allantoic fluid of eggs incubated in air and in 9% carbon dioxide*

(Values are also given for ammonium content in another series of experiments. Mean values. Number of samples in parenthesis.)

| Days of incubation | | 12 day | 13 day | 14 day | 15 day | 16 day | 17 day |
|---|---|---|---|---|---|---|---|
| pH | Air | 8·03 (6) | 7·96 (6) | 7·68 (6) | 7·26 (6) | 6·51 (6) | 6·97 (6) |
| | 9% $CO_2$ | 7·93 (6) | 7·79 (6) | 7·11 (6) | 7·98 (6) | 7·38 (6) | 6·99 (6) |
| Volume (ml) | Air | 6·2 (6) | 6·9 (6) | 5·8 (6) | 4·6 (6) | 2·6 (6) | 1·4 (6) |
| | 9% $CO_2$ | 6·2 (6) | 5·8 (6) | 5·8 (6) | 4·6 (6) | 6·0 (6) | 2·6 (6) |
| Titratable acidity ($\mu$-equiv.) | Air | −18 (6) | −18 (6) | −9 (6) | +3 (6) | +22 (6) | +9 (6) |
| | 9%($CO_2$ | −21 (6) | −16 (6) | −6 (6) | +4 (6) | 0 (6) | +9 (6) |
| Ammonium content ($\mu$-equiv.) | Air | — | — | 4·53 (6) | — | — | 11·18 (5) |
| | 9% $CO_2$ | — | — | 2·22 (6) | — | — | 13·76 (7) |

dioxide production. This accounts for the slight fluctuations in $P_{CO_2}$ seen in Fig. 1. Values for pH, ammonium ion content, titratable acidity and total volume of allantoic fluids are given in Table 3 for both normal embryos and those gassed with 9% carbon dioxide. The calcium content of embryos and whole egg contents are reported in Table 4. In these last series of experiments it was consistently found that the embryos exposed to high levels of carbon dioxide were less well developed than control animals.

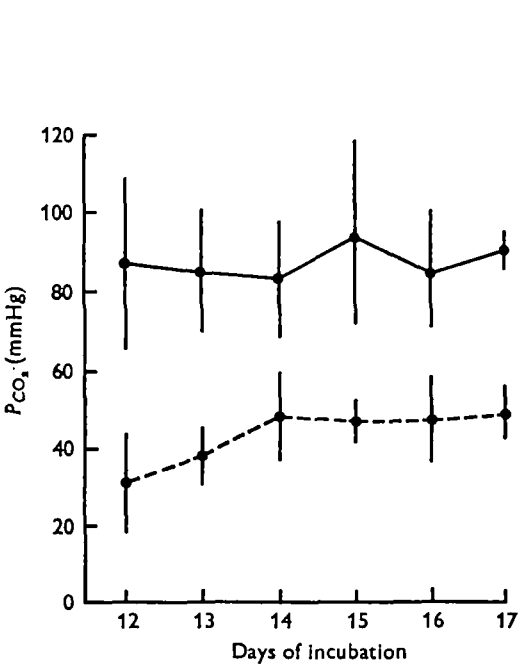


Fig. 1

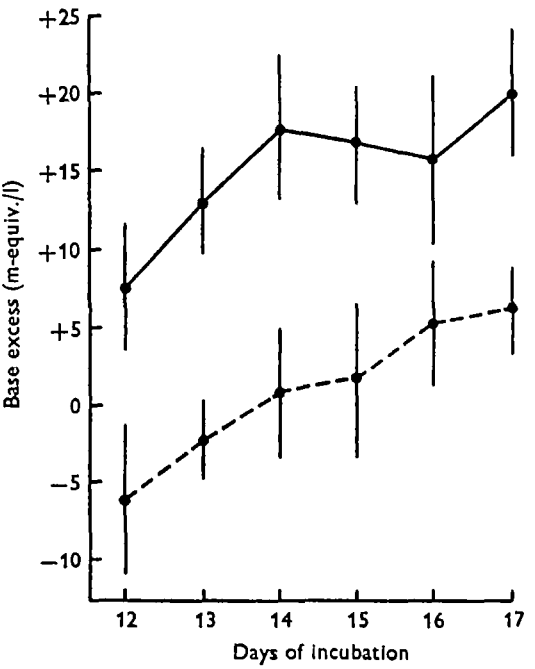


Fig. 2

Fig. 1. Changes in blood $P_{CO_2}$ during normal incubation (broken line) and during incubation in 9% carbon dioxide (solid line). Vertical lines represent standard deviations about the mean.

Fig. 2. Changes in base excess of the blood of embryos during normal incubation (broken line) and during incubation in 9% carbon dioxide (solid line). Vertical lines represent standard deviations about the mean.

This may be because the two incubators provided slightly different temperatures and thus produced slightly different rates of development. Alternatively it may be that high levels of carbon dioxide retard development slightly, but we have no evidence to support this. In practical terms it means that the control and experimentally treated embryos were at slightly different stages of development. This is shown in Fig. 3 where the length of the third toe has been plotted as an indication of stage of development (Hamburger & Hamilton, 1951). It is important to note, however, in discussing the results, that any slight delay in the development of the experimental animals would not be a critical factor in explaining any of the observed physiological differences in acid-base balance. Thus back dating any of the readings for experimental embryos by as much as one whole day (which overcompensates for any differences shown in Fig. 3) does not influence any of the trends shown in Figs. 1 and 2 or Tables 2–4.

Table 4. *Calcium contents of embryos and egg contents during normal incubation and after exposure to* 9% *carbon dioxide*

(Values are means ± S.D. (Number of samples in parenthesis).)

| | Wet weight (g) | Calcium content (total mg) | Calcium concentration (mgCa/g. wet wt) |
|---|---|---|---|
| 12-day embryos | | | |
| Controls | 4·71 ± 0·54 (6) | 1·43 ± 0·16 (6) | 0·305 ± 0·039 (6) |
| Experimental | 4·31 ± 0·86 (6) | 1·61 ± 0·39 (6) | 0·376 ± 0·070 (6) |
| 17-day egg contents | | | |
| Controls | 37·55 ± 2·64 (6) | 64·18 ± 3·22 (6) | 1·71 ± 0·10 (6) |
| Experimental | 36·44 ± 3·25 (6) | 59·08 ± 14·52 (6) | 1·65 ± 0·53 (6) |

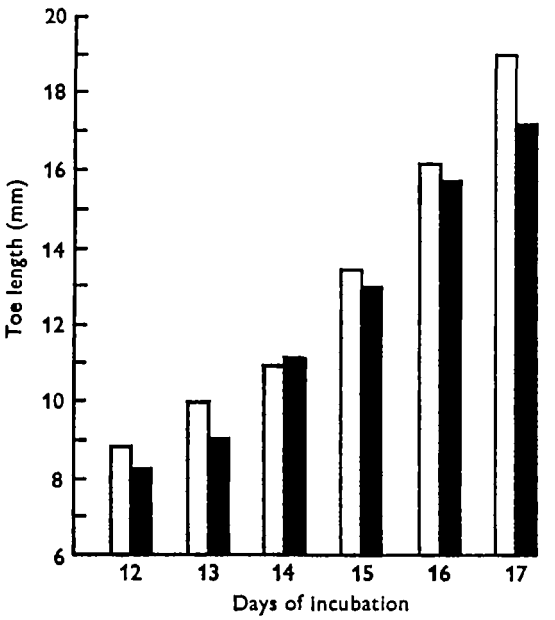


Fig. 3. Changes in mean length of third toe during normal incubation (white area) and during incubation in 9% carbon dioxide (black area).

## DISCUSSION

The results given in Table 2 and Fig. 2 show that the avian embryo is able to compensate for respiratory acidosis by a large and sudden increase in plasma bicarbonate levels. This compensation appears to be very rapid. Thus the embryos were given 9% carbon dioxide on day 9 and by day 12, when the first blood samples were taken, the gassed animals had an additional 13·9 m-equiv./l of base excess. Thereafter they showed little increase in this quantity and by day 17 they still showed a difference of only 14·0 m-equiv./l of base excess when compared with control animals. The compensation is therefore very rapid, but it is also incomplete and pH values tend to remain about 0·1 unit below normal throughout the experimental period.

There appear to be two main possibilities that could be invoked to explain the appearance of this additional bicarbonate. The first would involve a renal compensa-

tion in which the animal's kidney secretes protons into the urine. This could occur either in association with ammonia excretion to produce urinary ammonium ions, or alternatively it could be induced by a fall in pH in the renal tubules and a titration of the urinary buffers. An analysis of these possibilities is complicated by a number of peculiarities in the embryo. Thus any urine or nitrogen waste products formed by the embryo may be retained in the allantois of the embryo and not excreted from within the egg until hatching. Titrations of allantoic contents are therefore complicated, first by the reservoir of previously secreted urine which tends to make daily increments more difficult to determine, and secondly by the fact that the embryo resorbs the allantoic fluid and some of its ions throughout incubation. This latter phenomenon is clearly seen in the change in volume of allantoic fluid from a maximum on days 12–13 to the very small quantity found in the last few days of incubation (Table 3). This is a well-documented observation and together with the large fall in sodium and chloride ion content is good evidence for salt and water resorption from the allantois to the rest of the embryo (Stewart & Terepka, 1969). Thus renal activity is difficult to monitor during incubation. Despite these difficulties, however, it is possible to detect a fairly continuous fall in the pH of allantoic fluid during development (Table 3). This appears to be matched by a rise in ammonium ion content which would be expected if this was indeed caused by protons reacting with the more freely permeating ammonia. Estimates of the total proton excretion, however, must also involve back titrating the allantoic fluid to the pH of the blood from which it was presumably derived. These determinations of titratable acidity have been made to pH 7·4, but the titres obtained must be regarded as only approximate because of the continuous drift in titration caused by the strong buffering effect of uric acid and urates which are present in both solution and as solid waste. The estimates of titratable acidity, ammonium content and allantoic fluid volume are given in Table 3. Since the compensation to changes in blood pH appears to be largely complete by the time the first samples are obtained on day 12, it is at this stage that one must look for evidence of the underlying mechanism. The 12-day embryo has a plasma volume of about 1·2 ml (Barnes & Jensen, 1959) although the total extracellular volume is probably greater than this. An increase in base excess of 14 m-equiv./l in the plasma is therefore equivalent to at least 16·8 $\mu$-equiv. The difference in titratable acidity between normal and gassed embryos by day 12 is, however, virtually zero and in fact the urine is actually slightly more alkaline in the case of the hypercapnic animals. The difference in ammonium content of the allantoic fluids on day 14, when they were first measured, is 2·3 $\mu$-equiv. with the experimental animals again showing less excretion of protons. Both these responses are, perhaps, slightly surprising in that they show no indication of the removal of hydrogen ions from the embryo but perhaps even the reverse. They are, however, explicable on the basis that the observed large increase in plasma bicarbonate levels, if not caused by renal activity, would result in the ultrafiltration of large amounts of bicarbonate and thus an alkaline rather than an acid urine.

It therefore appears that there is no evidence to suggest that the source of the extra plasma bicarbonate in the gassed embryo is due to some renal mechanism. Recourse must therefore be made to a second postulate, namely that there is some other source of the extra base, and the most likely possibility is that this is obtained by the resorption of increasing amounts of eggshell minerals. This process presumably occurs during

the uptake of calcium from the eggshell by the chorioallantoic membrane (Simkiss, 1967), so the hypothesis could be briefly tested by analysing embryos and whole egg contents for the extra calcium which would presumably by associated with the extra bicarbonate. The results of this analysis are shown in Table 4. In the early 12-day samples the gassed embryos contained slightly more calcium than the controls although there was a good deal of variation and the increase amounted to only 0·18 mg. At 17 days analyses of total egg contents showed slightly more calcium in the control eggs although the total quantities involved are now large, i.e. $64{\cdot}18 \pm 3{\cdot}22$ mg. If one takes the 12-day samples then the quantity of shell which would have to be resorbed to release 16·8 $\mu$-equiv. of extra bicarbonate can easily be calculated and would only release 0·34 mgCa. This is within the range of experimental analysis and error for the 12-day samples and would be completely swamped in the analysis of total calcium in the 17-day samples where it would be only about 0·5% of the total.

It therefore appears most likely that the extra bicarbonate in the plasma of gassed embryos is derived from the shell. It was not possible in the present experiments, however, to demonstrate conclusively the increased amount of calcium which would be liberated at the same time. The mechanism for the resorption of the eggshell by the chorioallantoic membrane of the normal embryo remains something of a mystery. The elegant analyses and experiments of Terepka and his co-workers (Moriarty & Terepka, 1969; Stewart & Terepka, 1969; Terepka, Stewart & Merkel, 1969) have provided good evidence for the active transport of calcium ions, but it remains to be demonstrated as to whether shell resorption is also active or merely passive depending upon local conditions. The rapid incorporation of extra bicarbonate, the absence of a continually increasing response, and the incompleteness of the response are all similar to those observed in other animals containing calcareous deposits (Simkiss, 1968). It is therefore interesting to speculate whether the phenomenon is thus to a large extent simply a physical solubility response of the shell to increased carbon dioxide, or whether there are some common physiological mechanisms in these different situations.

SUMMARY

1. Embryos of the domestic fowl have been incubated in normal conditions and in an atmosphere of 9% carbon dioxide from day 9 onwards.

2. There is a rapid increase in blood bicarbonate and base excess when the embryos are exposed to carbon dioxide. The increase amounts to about 14 m-equiv./l and is relatively constant from day 12 onwards.

3. There is no detectable increase in titratable acidity or ammonium ion content of allantoic fluid which would indicate that the extra plasma bicarbonate ions were formed by renal activity.

4. It is suggested that the extra bicarbonate of the blood is derived by resorption of eggshell minerals.

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