Gas Exchange and Gas Permeation of Duck Eggs by the Use of Oxygen Analyzing Apparatus

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Apparatus has been devised for the determination of the gas exchange and permeation of fertile duck eggs. The oxygen uptake of fertile eggs was measured continuously, and lead the condition of the embryos whether they were dead or alive during incubation. The apparatus for the determination of gas permeation simultaneously determine oxygen, water and carbon dioxide which permeate through the eggshell. By the record of the oxygen consumption for the fertile eggs, the development of the embryos in the course of incubation could be traced. Relations between the gas permeation of the fertile eggs and the growth of the eggshells was also revealed in connection with the oxygen demand.

Keywords Direct oxygen determination, duck embryo, gas permeation, gas exchange, simultaneous determination

Since Romijn et al. determined the amount of oxygen absorbed by fertile eggs by gas analysis using a manometer, many reports have been made on the determination of the oxygen consumption of fertile eggs. However, all the methods reported so far rely on the analysis of the gas in the air space of eggs which is corrected from the eggs by breaking the eggshell. Thus it has been assumed desirable to invent an apparatus which allows long-term and direct measurement of the oxygen consumption. The manometer or the electrode is not suitable for the present investigation which requests high stability and simultaneous determination of oxygen, water and carbon dioxide. The present authors have improved the induction period meter which have previously been designed, and applied it to the consecutive determination of oxygen consumption of the incubated fertile eggs. The authors have also modified the direct oxygen determination apparatus and made simultaneous measurements of oxygen, water and carbon dioxide which permeate through eggshells.

Experimental

Determination of the oxygen consumption

Figure 1 shows the oxygen absorption measuring apparatus which allows the measurement of the oxygen uptake of the fertile eggs for a long period. Figure 2 is a 270 ml cell which holds the duck egg. Eggs used were obtained from Khaki Canbell ducks. The eggs were 52–55 g in weight and about 50 ml in volume before incubation. An egg was placed in the cell of the oxygen absorption measuring apparatus. A 5 g portion of the granular potassium hydroxide was placed in the lower part of the cell which absorbed carbon dioxide evolved by the egg. The cell was then transferred to an incubator containing 2% sodium sulfate (electrolytic solution) and kept at 38±0.01°C. When the partial pressure of oxygen in the cell was noticed to get reduced as the result of the oxygen uptake of the embryo, the electrolytic solution was introduced to the absorption tube and allowed to contact with the platinum electrode within the absorption tube. By the latter procedure, the electrolysis circuit was closed, and evolved oxygen from the positive electrode. The quantity of electricity was converted to a digital count.

![Fig. 1 Oxygen absorption measuring apparatus. 1. thermostat(38±0.01°C); 2. platinum electrode(1φ×10 mm); 3. electrolytic solution(sodium sulfate); 4. absorption tube(4 mm i.d.); 5. CO₂ absorption agent (potassium hydroxide); 6. cell; 7. fertile egg; 8. power supply(30 V); 9. resistance(5 kΩ); 10. V/F converter (RC 4151); 11. digital counter(MH 730); 12. D/A converter(ADPAC 80); 13. recorder.](image-url)
through a resistor and a V/F converter, and integrated. The integration values were continuously recorded on a recorder through a D/A converter. One count corresponded to 1.82 µl of oxygen at 38°C. The reproducibility of the instruments was ±2.9%. With this instrument, the oxygen consumption of the fertile egg was automatically recorded for 28 consecutive days of incubation.

**Gas permeation measurement**

Figure 3 shows the outline of the measuring apparatus. An eggshell was fixed to the holder of gas permeation apparatus with paraffin. Purified nitrogen was introduced as the carrier gas into the holder, and carried oxygen, water and carbon dioxide which had permeated from the atmosphere into the inside of the eggshell to the combustion furnace containing platinum-carbon at 900°C. The flow rate of nitrogen was 10 ml/min at 1.1 atm. In the furnace, all the gases were completely converted into carbon monoxide. Then, carbon monoxide was oxidized to carbon dioxide by iodine pentoxide. The carbon dioxide content was determined with an electric current titration apparatus. The amount of oxygen, water and carbon dioxide could be separately determined by combining a water absorption tube (magnesium perchlorate), or carbon dioxide absorption tubes (sodium hydroxide) with the apparatus. The gas permeation at both ends of egg was measured sequentially at 0, 7, 14, 21 and 28 days of incubation under the following conditions: flow rate of nitrogen, 10 ml/min; temperature, 30°C; humidity, 55%RH; and measuring time, 5 minutes. The reproducibility of the instrument was ±0.3%.

**Observation of the surface of an eggshell and determination of its thickness**

The structure of an eggshell was observed with a scanning electronic microscope (MSM-9, Akashi Co. Ltd.) on the days 0, 7, 14, 21 and 28 of incubation at a magnification of ×700.

**Results**

The oxygen consumption of the duck embryos was found as follows.

Figure 4 shows the cumulative oxygen consumption and the daily oxygen consumptions (n=4) for the 28 day incubation period. With the incubation time, the oxygen consumption increased up to 8.1 liters on the 28th days of incubation. The oxygen uptake of the duck embryos began on the 5 or 6 days of incubation, then linearly increased at a rate of 67 ml/d from the 10th day to 22nd day. Then, the rate of the oxygen uptake got reduced from 23rd day to 26th, but increased rapidly again from 26th to 28th day.

The gas permeation of the eggshells showed the following results.
Figure 5 shows the permeation rates of oxygen, water and carbon dioxide at the blunt end of the fertile duck eggs on days 0, 7, 14, 21 and 28 of incubation. The permeated amounts of oxygen, water and carbon dioxide increased with the elapse of incubation time and on day 28, large permeation rates were obtained for, in the order, water, oxygen and carbon dioxide. The permeation rate of water was as low as 0.31 g m\(^{-2}\) d\(^{-1}\) atm\(^{-1}\) at the early stage of incubation but increased by 3.84 g m\(^{-2}\) d\(^{-1}\) atm\(^{-1}\) from the day 14 to the day 28.

The structure and thickness of eggshell were found following.

Photomicrographs of the surface (cuticula) of the blunt ends of eggshells are given in Fig. 6. As compared with the eggshell surface on day 0, many cracks of the cuticular structure were observed on the day 21 of the incubation and then the cracks became large on the day 28. As Table 1 shows, the thickness of eggshells at the blunt end reduced with the elapse of the incubation time and the largest reduction of 74 µm was observed from the day 14 to the day 21. On the day 21 of incubation, the thickness of the blunt end was 30 µm thinner than the pointed end. When compared with the pointed end on the day 21, the blunt end showed large gas permeation rates for oxygen, water and carbon dioxide; especially, the permeation rates of oxygen and water of the blunt end were 1.8-fold and 2.8-fold larger than those of the pointed end (Table 2).

Discussion

The results of the measurement of the present investigation show evident correlation with the knowledge on the development of the egg and suggest the usefulness of the

<table>
<thead>
<tr>
<th>Incubation/d</th>
<th>Thickness/µm</th>
<th>Reduction of thickness/µm</th>
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<tbody>
<tr>
<td>0</td>
<td>422 ± 4</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>405 ± 5</td>
<td>17 (day 0-7)</td>
</tr>
<tr>
<td>14</td>
<td>372 ± 7</td>
<td>32 (day 7-14)</td>
</tr>
<tr>
<td>21</td>
<td>298 ± 6</td>
<td>74 (day 14-21)</td>
</tr>
<tr>
<td>28</td>
<td>287 ± 4</td>
<td>11 (day 21-28)</td>
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</table>

| Gas permeation rates of duck eggshell on day 21 of incubation at 30°C (n=4) |
|-------------------------------|-----------------|-----------------|
|                               | Permeation/µm² d⁻¹ atm⁻¹ |                |
|                               | O₂              | H₂O             | CO₂             |
| Blunt end                     | 2.07 ± 0.15     | 1.45 ± 0.29     | 0.53 ± 0.14     |
| Pointed end                   | 1.17 ± 0.20     | 0.51 ± 0.12     | 0.05 ± 0.09     |

Fig. 5 Eggshell transmission rates of O₂, H₂O, and CO₂ during 28-day incubation time (n=4). ——: O₂; ——: H₂O; ——: CO₂.

(1) Day 0 (2) Day 21

Fig. 6 Photomicrographs of eggshell surface (×700)
device which has been invented by the present investigation. The results of the oxygen measurement refers to the start of the oxygen uptake at the 4th and 5th day of incubation which must certainly be correlated with the change in the development\textsuperscript{14}, the embryonic metabolism of the formation of organs. The increase of oxygen consumption on the day 14 accords the start of skeletal formation in which development of the blood vessels around the yolk sac has been completed. The depressed increase of the oxygen consumption on the day 22–25 may refer to the preparatory time for pipping following completion of the skeletal system. The remarkable rise in the oxygen consumption on the day 26–28 corresponds to the period when pipping commences following the change from the allantoic respiration to the pulmonary one and the egg is hatchable. The gas permeation rate of the eggshell shows sizable increase from the day 14 on, which correlates remarkably with the changes in the eggshell thickness and the cuticular structure. From the fact that the eggshell thickness is reduced remarkably from the day 14 to the day 21, calcium in the eggshell is believed to have transferred to the embryo and participated in skeletal formation.\textsuperscript{15–17} Furthermore, it is assumed that the increased amounts of oxygen and water which have permeated through the increased cracks among of the cuticular structures in the eggshell satisfy the oxygen demand for the embryonic growth.

The oxygen absorption measuring apparatus can automatically determine the oxygen uptake of the fertile egg for a long period, and can distinguish the fertile eggs and the living embryos from the nonfertile eggs and the dead embryos at an early stage of incubation. The gas permeation measuring apparatus presented in this report can determine oxygen, water and carbon dioxide simultaneously in a short time at the atmospheric pressure, and the values obtained with this apparatus clearly show the gas exchange of the eggs which refer to the development of the incubated fertile eggs.

References


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