Analysis of the air cell gas-arterialized blood Po difference of chicken eggs with respect to widely altered shell conductance

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Analysis of the air cell gas-arterialized blood P_{O_2} difference of chicken eggs with respect to widely altered shell conductance

若 山 博 司・小 師　　隆・鈴 木 幸 司・田 澤　　皓

Hiroshi WAKAYAMA, Takashi KOMORO, Yukinori SUZUKI and Hiroshi TAZAWA

Abstract

The gas exchange of chicken eggs takes place by molecular diffusion. The diffusion barrier between ambient atmosphere and erythrocyte hemoglobin of the gas exchanger is conveniently divided into two parts by the air cell formed at the blunt end; *i.e.*, outer barrier (mainly the porous eggshell) and inner barrier (the chorioallantoic membrane). In contrast to the alveolar-arterial P_{O_2} difference in the lungs, the difference of P_{O_2} between the air cell and the arterialized blood in the allantoic vein ($\Delta P_{A_{O_2}} \cdot Pa_{O_2}$) is large in chick embryos. In the present study was analyzed the $\Delta P_{A_{O_2}} \cdot Pa_{O_2}$ with respect to widely altered diffusive conductance of the shell, diffusing capacity of the chorioallantoic membrane and physiological shunt in the allantoic circulation (allantoic shunt).

The shell diffusive conductance (G_{O_2}) was altered from the beginning of incubation, and the O_2 consumption ($\dot{M}_{O_2}$) was measured on day 16. The $\dot{M}_{O_2}$ increased hyperbolically with increasing G_{O_2}, reached maximum at control values of G_{O_2} and decreased with further increasing G_{O_2}. From G_{O_2} and $\dot{M}_{O_2}$, the air cell P_{O_2} was determined. The $\Delta P_{A_{O_2}} \cdot Pa_{O_2}$ was increased in eggs with augmented G_{O_2} (from about 50 torr in control eggs to 70 torr in conductance-increased eggs). The diffusing capacity and allantoic shunt which produce a given $\Delta P_{A_{O_2}} \cdot Pa_{O_2}$ were estimated employing a microcomputer performing the Bohr integration procedure so that a calculated Pa_{O_2} agreed with measured Pa_{O_2}. The allantoic shunt is not more than 20%; 10% is likely. The diffusing capacity becomes maximum in intact (control) eggs and is decreased at both lowered and augmented G_{O_2}. In control eggs, the resistance of the inner barrier to O_2 diffusion is about 1.7-fold that of the shell which agrees with the previous reports.

1. Introduction

In contrast to the convective transport of respiratory gases in animals having lungs, the gas exchange of avian eggs takes place by diffusive transport. The diffusion barrier lying between ambient atmosphere and the capillary blood of the gas exchanger (vascularized chorioallantoic membrane) is conveniently divided into two parts by the air space in the fibrous shell membranes. The outer barrier comprises the porous eggshell and outer shell membrane. The conductance for gas diffusion is mainly attributed to the shell, thus it is determined by porosity and thickness of the shell. Between the outer and inner shell membranes is formed the air cell at the blunt end which is assumed to substitute the air space. The O_2 in the air cell is transferred through the

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inner shell membrane and the chorioallantoic membrane to the capillary blood.　These two membranes constitute the inner diffusion barrier and its diffusive conductance includes the gas reaction with blood in the chorioallantoic capillary.　While the diffusive conductance of the outer barrier corresponds to the alveolar ventilation multiplied by the capacitance coefficient in the convective transport system (Rahn and Paganelli, 1982), the air cell is an equivalent to the alveolar space and the conductance of the inner barrier corresponds to the diffusing capacity of the lung. In contrast with the alveolar-arterial P_{O_2} difference in the lung, the difference of P_{O_2} between the air cell and the arterialized blood in the allantoic vein is large in chick embryos (Piiper *et al.*, 1980).　Because in chicken eggs the diffusive conductance of the outer barrier can be altered widely, we analyzed the P_{O_2} difference between the air cell and arterialized blood ($\Delta P_{A_{O_2}} \cdot P_{a_{O_2}}$) with respect to widely altered diffusive conductance of the shell, the diffusing capacity of the chorioallantoic membrane and physiological shunt in the allantoic circulation (referred to as allantoic shunt).

2．Methods and materials

Fertile chicken eggs were measured for length (L, cm) and maximum breadth diameter (B, cm) to estimate the fresh egg mass (W, g) using the equation of $W = 0.5632 B^2 L$ (Romanoff and Romanoff, 1949).　Eggs were divided into three groups at random; the first group eggs had the eggshell of the sharp end covered with epoxy cement for a quarter to half the length of the egg (referred to as conductance-decreased eggs), the second group eggs had the eggshell covering the air cell was removed over a width of 3 to 10 mm across (conductance-increased eggs) and third group eggs remained intact (control eggs).　These eggs were incubated at 38°C and approximately 55% of relative humidity for 16 days.

On day 14-15 of incubation, the water vapor conductance of the shell was determined with 'calibrated egg' technique reported by Tullett (1981).　The water vapor conductance, G_{H_2O} in mg·day^{-1}·torr^{-1}, of the infertile egg which was subsequently used as 'calibrated egg' was determined first by measuring weight loss for a given period in dry atmosphere (buried in silica gel) at 38°C.　The G_{H_2O} of an experimental egg was then determined from the ratio of water loss rate of experimental egg to that of calibrated egg multiplied by G_{H_2O} of the calibrated egg.　For actual measurements, 4 calibrated eggs were used and kept together with the experimental eggs in the same incubator.　The G_{H_2O} multiplied by 1.06 (the ratio of volume to molecular weight of water vapor multiplied by the diffusion coefficient ratio of water vapor to oxygen at 38°C) provides the conductance for O_2 (G_{O_2} in ml STP·day^{-1}·torr^{-1}).　For standardization, G_{O_2} was divided by

fresh egg mass to provide specific conductance for O_2, g_{O_2} in ml STP·day^{-1}·torr^{-1}·(g$_{egg}$)$^{-1}$.

On day 16 of incubation, the O_2 consumption of individual eggs was determined using a modified Scholander and Edwards respirometer (Scholander and Edwards, 1942) submerged in 38°C water bath.   The respirometer consisted of two equal-size aclyric chambers by U-shaped water-filled glass manometer.   As an embryo within experimental egg, placed in one chamber along with CO_2 absorber, consumed O_2, the level of water in the manometer was displaced.   The displacement was corrected by injecting pure O_2 into the chamber containing the experimental egg.   The O_2 consumption ($\dot{M}_{O_2}$ in ml STPD·day^{-1}) was calculated from the volume of O_2 injected in a given time.

After measurement $\dot{M}_{O_2}$, each egg was subjected to blood sampling from the allantoic vein as stated previously (Tazawa, 1971).   Immediately after collection of 0.4 ml-blood into the Hamilton glass syringe (No. 750), the blood was measured for P_{O_2} and pH with an Instrumentation Laboratory blood gas analyzer (type 213 and 326).

Analysis of the air space gas-arterialized blood P_{O_2} difference

Air cell P_{O_2} (PA_{O_2})   In the diffusive transport in chicken eggs, the O_2 consumption ($\dot{M}_{O_2}$) is determined by the shell conductance for O_2 (G_{O_2}) multiplied by P_{O_2} difference between the environment and the air cell ($PI_{O_2} - PA_{O_2}$).   Because the effective P_{O_2} of the environment (PI_{O_2}) is known, the air cell P_{O_2} is calculated from the $\dot{M}_{O_2}$ and G_{O_2} measured for each egg as follows,

$$PA_{O_2} = PI_{O_2} - \dot{M}_{O_2} / G_{O_2} \tag{1}$$

Estimation of diffusing capacity (D_{O_2}) and allantoic shunt ($\dot{q}_{sh}$)   The $\Delta PA_{O_2} \cdot Pa_{O_2}$ is attributed to the diffusing capacity of the inner diffusion barrier (D_{O_2}) and the physiological shunt from the allantoic artery to the vein in the allantoic circulation (allantoic shunt, $\dot{q}_{sh}$ in %) (Piiper *et al.*, 1980).   The D_{O_2} and $\dot{q}_{sh}$ were estimated so that they produce a given $\Delta PA_{O_2} \cdot Pa_{O_2}$.   The sequence of estimation procedure using microcomputer is indicated as follows (and Fig. 1).

(1) D_{O_2} and $\dot{q}_{sh}$ are assigned temporary values by reference to the previous reports.

(2)   Mean capillary blood P_{O_2} ($P\bar{c}_{O_2}$) is calculated from $\dot{M}_{O_2}$ and D_{O_2} as follows,

$$P\bar{c}_{O_2} = PA_{O_2} - \dot{M}_{O_2} / D_{O_2} \tag{2}$$

(3)   End-capillary blood P_{O_2} (Pc_{O_2}) is estimated from the Bohr integration so that the area under the O_2 dissociation curve (ODC), which includes the dissolved O_2, from P_{O_2} of the allantoic
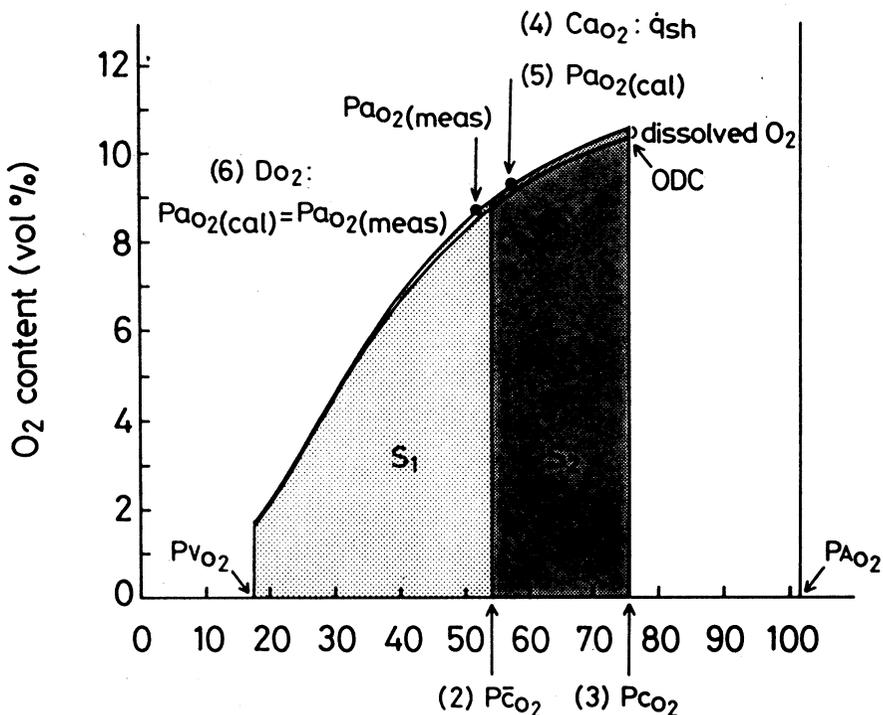
Fig. 1. Sequence of numerical analysis of diffusing capacity (D$_{O_2}$) and allantoic shunt ($\dot{q}_{sh}$) (Nos. (1)–(6)).　Ca$_{O_2}$= blood O$_2$ capacity, ODC=oxygen dissociation curve, PA$_{O_2}$=air space O$_2$ tension, Pa$_{O_2}$ (cal)=calculated value of arterialized blood O$_2$ tension, Pa$_{O_2}$ (meas) = measured value of arterized blood O$_2$ tension, Pc$_{O_2}$=end-capillary blood O$_2$ tension, Pc̄$_{O_2}$=mean-capillary blood O$_2$ tension, and Pv$_{O_2}$=mixed blood O$_2$ tension in the allantoic artery.　See text for more detailed explanation.

artery blood (Pv$_{O_2}$) to Pc̄$_{O_2}$ (S$_1$) becomes identical with that from Pc̄$_{O_2}$ to Pc$_{O_2}$ (S$_2$).　The ODC reported previously for 16-day embryos (Tazaja *et al.*, 1976) is,

$$log\, P_{O_2}=4.932-0.448\,pH+0.394\,log\,S_{O_2}\,/\,(100-S_{O_2}) \tag{3}$$

where pH determind for each embryo is used.　The Pv$_{O_2}$ which is hyperbolically related to the shell conductance (Nakazawa and Tazawa,1987) is estimated from individual g$_{O_2}$ as follows,

$$Pv_{O_2}=20.6-0.53\,g_{O_2}^{-1} \tag{4}$$

The O$_2$ content for a given blood P$_{O_2}$ is thus estimated from

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$$C_{O_2} = O_2\ capacity \cdot S_{O_2} / 100 + \alpha_{O_2} \cdot P_{O_2} \tag{5}$$

where O_2 capacity has been reported to be 12 vol % for 16-day embroyos (Tazawa and Mochizuki, 1977) and O_2 solubility coefficient (α_{O_2}) of 0.00311 ml·(100 ml·torr)$^{-1}$ is used.

(4) The arterialized blood O_2 content in the allantoic vein (Ca_{O_2}) is estimated by taking $\dot{q}_{sh}$ into consideration as follows,

$$Ca_{O_2} = Cc_{O_2} - (Cc_{O_2} - Cv_{O_2}) \cdot \dot{q}_{sh} \tag{6}$$

where end-capillary O_2 content (Cc_{O_2}) and mixed arterial O_2 content in the allantoic artery (Cv_{O_2}) are estimated from ODC and dissolved O_2 content for a given P_{O_2}.

(5) Then, Pa_{O_2} is calculated from Ca_{O_2} and ODC.
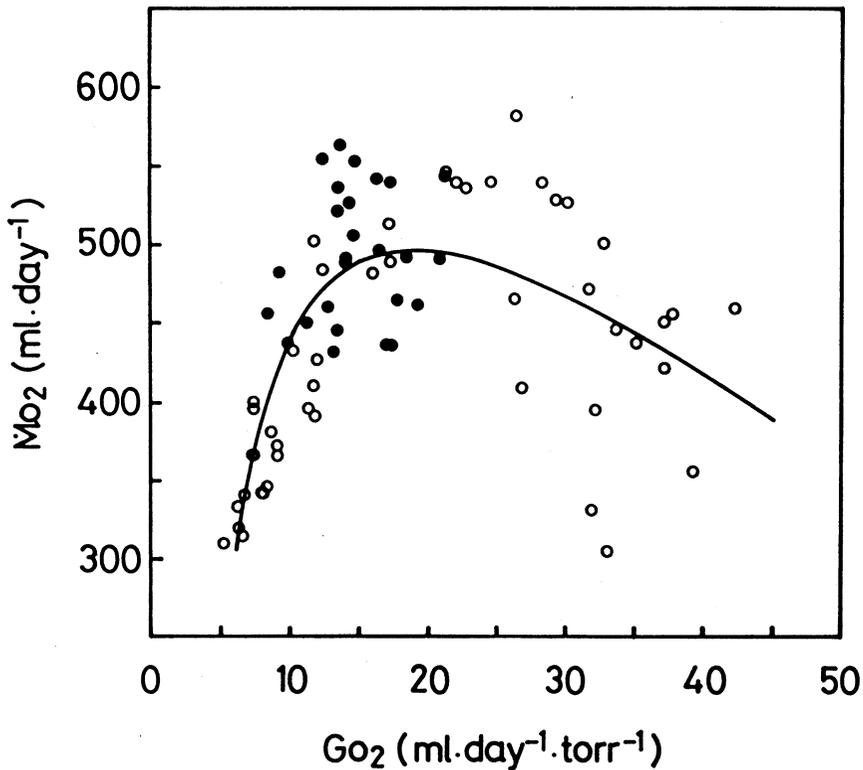
Fig. 2. Oxygen consumption of control (closed circles), conductance-decreased and conductance-increased eggs measured on day 16 of incubation.

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(6) Procedures (2) − (5) are repeated altering D_{O_2} until Pa_{O_2} calculated as above equals to measured Pa_{O_2} or the difference between the two values becomes smaller than a given difference. The conductance obtained as above is the D_{O_2} producing $\Delta PA_{O_2} \cdot Pa_{O_2}$ is examined by repeating the above procedure altering $\dot{q}_{sh}$ while D_{O_2} is kept constant.

3. Results

The O_2 consumption measured for 27 control, 24 conductance-decreased and 23 conductance-increased eggs is plotted for individual conductances in Fig. 2. The control eggs are shown by closed circles. For widely altered conductances, the $\dot{M}_{O_2}$ increases hyperbolically with increasing G_{O_2} until conductance reaches control range and then decreases. The best-fit regression curve is expressed by the following equation,
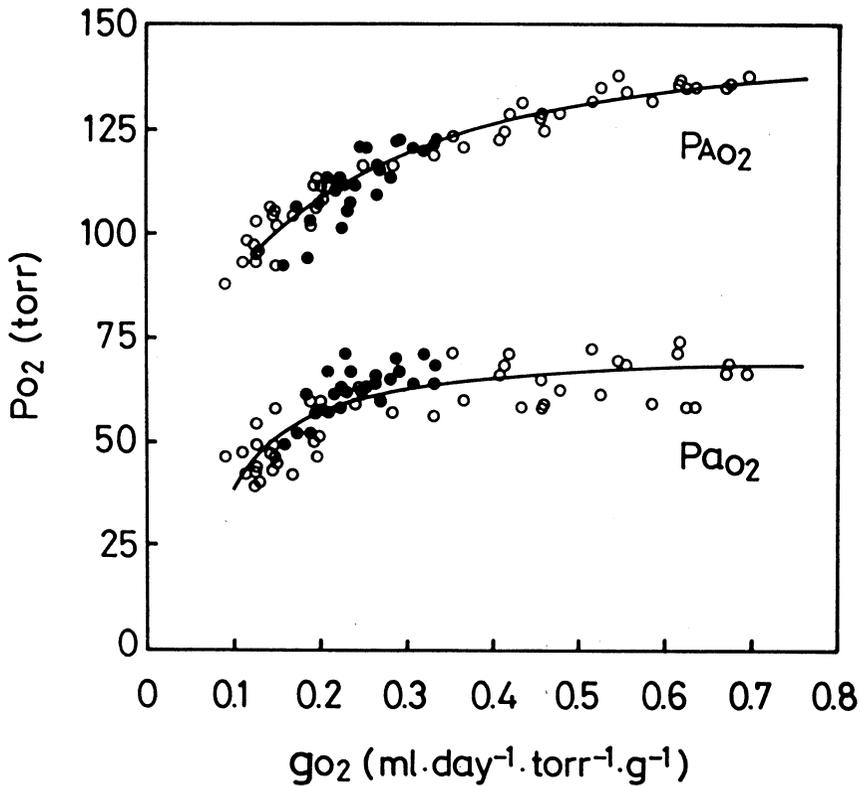
Fig. 3. Oxygen tensions measured for arterialized blood (Pa_{O_2}) and calculated for air space (PA_{O_2}) of 16-day-old embryos with widely altered shell conductances. Control eggs are shown by closed circles.
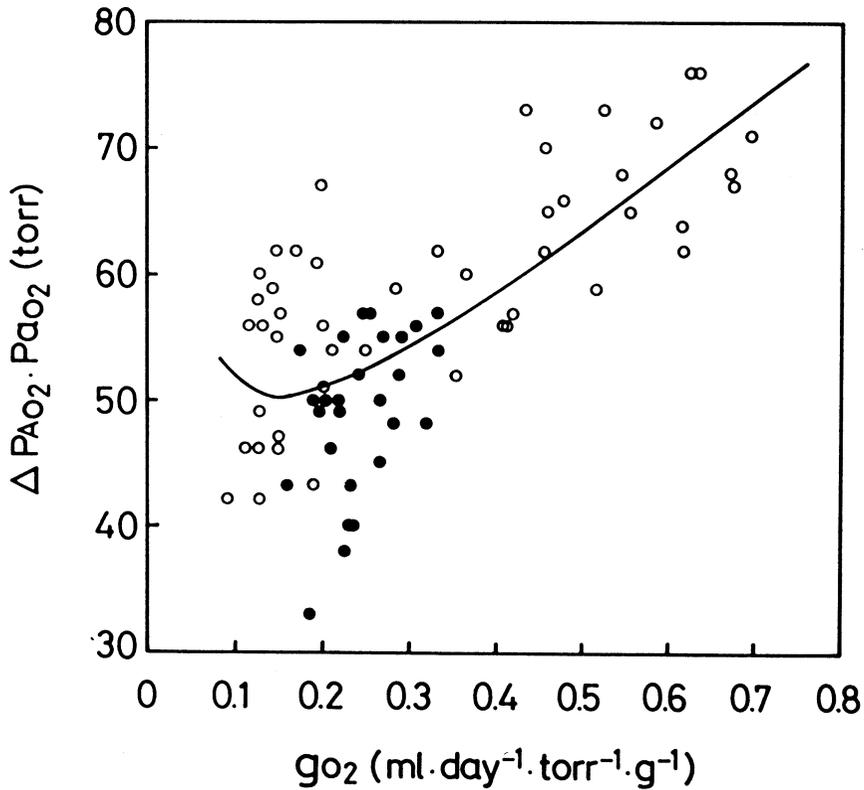
Fig. 4. Oxygen tension differences between air cell gas and arterialized blood of 16-day-old embryos shown in Fig. 3.

$$\dot{M}_{O_2} = 768 - 2567 \, G_{O_2}^{-1} - 7.17 \, G_{O_2} \tag{7}$$

where the coefficients of the 2nd and 3rd terms are significant by the Fisher test (F = 68.6 and 33.0, respectively).   The decrease in $\dot{M}_{O_2}$ at large conductances is attributed to the small size of embryos, caused by excess water loss (Okuda and Tazawa, 1987).

Fig. 3 represents the P_{O_2} of arterialized blood collected from the allantoic vein (Pa_{O_2}) and the air cell P_{O_2} (PA_{O_2}) calculated using eq. (1).   They are plotted for individual g_{O_2}.   The quadratic regression equations of inverse g_{O_2} expressed by

$$Pa_{O_2} = 72.0 - 2.86 \, g_{O_2}^{-1} - 0.033 \, g_{O_2}^{-2} \tag{8}$$

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0.25

do_2 (ml·day^{-1}·torr^{-1}·g^{-1})

0.2

0.15

0.1

0.05

0

20

10%

0

0　0.1　0.2　0.3　0.4　0.5　0.6　0.7　0.8

go_2 (ml·day^{-1}·torr^{-1}·g^{-1})
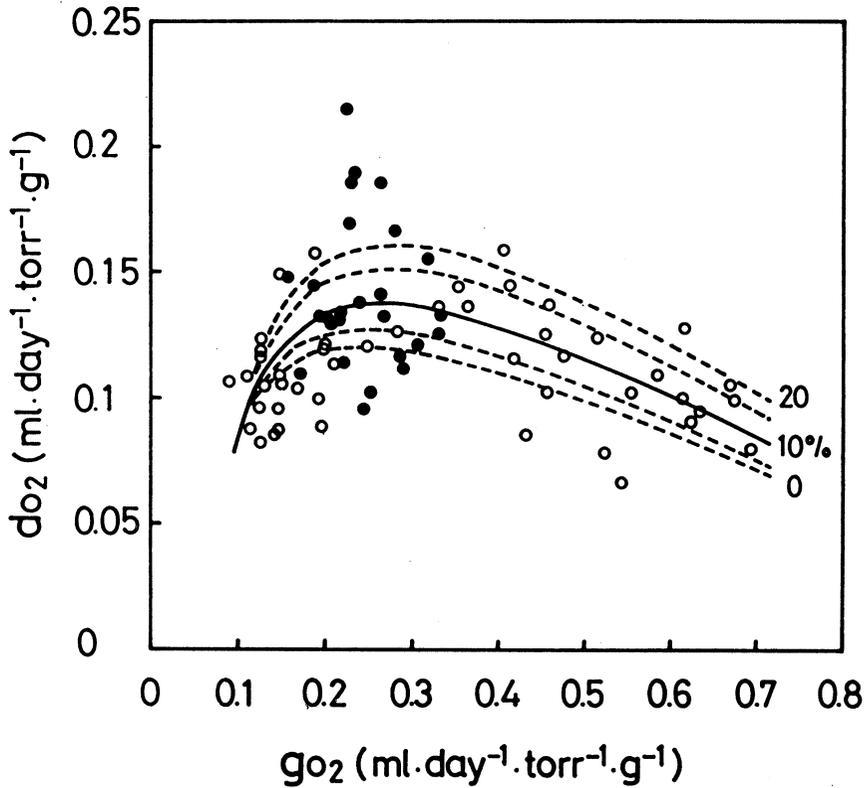
Fig. 5. Diffusing capacity (standardized by egg mass) estimated so that it produces individual $\Delta P_{A_{O_2}} \cdot P_{a_{O_2}}$ shown in Fig. 4 and 10% allantoic shunt. The solid curve is drawn from the regression equation, and dashed lines indicate the regression equation calculated for $\dot{q}_{sh}$ of 0, 5, 15 and 20%.

$$P_{A_{O_2}} = 150.0 - 11.09\, g_{O_2}^{-1} + 0.540\, g_{O_2}^{-2} \tag{9}$$

give a good fit for the relation between O_2 tension and conductance. The significance of the regression equation which becomes quadratic is discussed elsewhere (Okuda and Tazawa, 1987). The $\Delta P_{A_{O_2}} \cdot P_{a_{O_2}}$ increases at increased and decreased g_{O_2} (Fig. 4). The relation between $\Delta P_{A_{O_2}} \cdot P_{a_{O_2}}$ and g_{O_2} is expressed by

$$\Delta P_{A_{O_2}} \cdot P_{a_{O_2}} = 34.3 + 1.18\, g_{O_2}^{-1} + 53.9\, g_{O_2} \tag{10}$$

where 3rd term is significant by F-test (F = 30.62, while for 2nd term F = 2.64).

Fig. 5 shows the diffusing capacity and allantoic shunt which produce the $\Delta P_{A_{O_2}} \cdot P_{a_{O_2}}$ plotted

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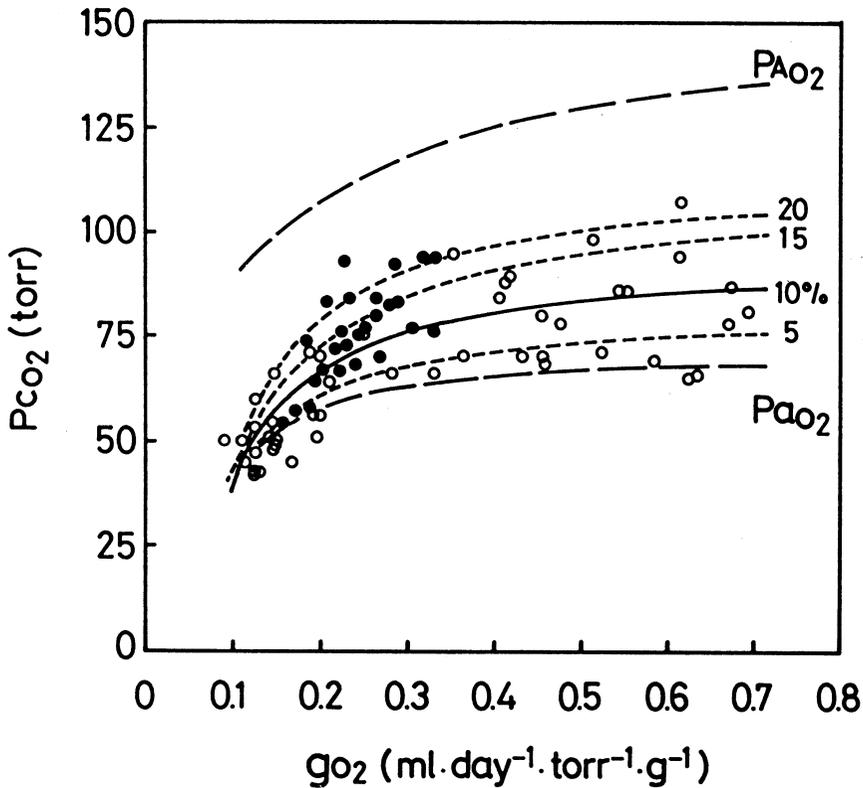
Fig. 6. End-capillary blood P_{O_2} of individual eggs having diffusing capacity shown in Fig. 5 and allantoic shunt of
10%. Solid curve is drawn from the best-fit regression equation, and dashed curves indicate the regres-
sion equation calculated for $\dot{q}_{sh}$ of 5, 15 and 20%. The regression curves for P_{AO_2} and P_{aO_2} shown in
Fig. 3 are also presented (broken lines).

for individual g_{O_2} in Fig. 4. The diffusing capacity is standardized by fresh egg mass (*i.e.*, specif-
ic diffusing capacity, d_{O_2} in ml STP·day^{-1}·torr^{-1}·$(g_{egg})^{-1}$) for comparison with g_{O_2} of the outer
barrier. Each plot indicates d_{O_2} when $\dot{q}_{sh}$ is 10% and the solid curve shows the best-fit regres-
sion equation expressed by

$$d_{O_2} = 0.237 - 0.013 \, g_{O_2}^{-1} - 0.191 \, g_{O_2}$$

where the hyperbolic and linear parameters are significant ($F = 19.6$ and $F = 23.5$, respectively).
For $\dot{q}_{sh}$ of 0, 5, 15 and 20% ($N = 74$, 74, 66 and 48, respectively), only the regression curves are
shown (dashed lines).

Fig. 6 represents the end-capillary blood P_{O_2} (Pc_{O_2}) plotted for individual g_{O_2} values when the

allantoic shunt is assumed to be 10% and the diffusing capacity has a value shown in Fig. 5. For $\dot{q}_{sh}$ of 5, 15 and 20%, the regression curves only are presented (dashed lines). The regression curves for $P_{A_{O_2}}$ and $P_{a_{O_2}}$ shown in Fig. 3 are also presented (broken lines).

4. Discussion

In contrast to the alveolar-arterial P_{O_2} difference in the healthy lung, the $\Delta P_{A_{O_2}} \cdot P_{a_{O_2}}$ of chicken eggs is large (Figs. 3 and 4); the average $\Delta P_{A_{O_2}} \cdot P_{a_{O_2}}$ of intact control eggs is 49 ± 6 (SD) torr (N = 27). It becomes large when the shell conductance is increased from the control ranges (Fig. 4), suggesting that the diffusing capacity of the inner barrier decreases and/or the allantoic shunt increases.

For eggs incubated normally for 16 days, the D_{O_2} has been reported to be 6.0 $\mu l \cdot min^{-1} \cdot torr^{-1}$ (Tazawa and Mochizuki, 1976), 4.9 $\mu l \cdot min^{-1} \cdot torr^{-1}$ (Tazawa and Mochizuki, 1977), 7.0 $\mu l \cdot min^{-1} \cdot torr^{-1}$ (Piiper et al., 1980) and 6.8 $\mu l \cdot min^{-1} \cdot torr^{-1}$ (Wangensteen and Weibel, 1982), and the D_{CO} to be 5.1 $\mu l \cdot min^{-1} \cdot torr^{-1}$ (Bissonnette and Metcalfe, 1978). As to the allantoic shunt, Piiper et al. (1980) reported it to be 10–15%. In order to produce $\Delta P_{A_{O_2}} \cdot P_{a_{O_2}}$ calculated for the present control eggs (N = 27), the D_{O_2} has to be larger than 4 $\mu l \cdot min^{-1} \cdot torr^{-1}$ (since the averaged egg mass is about 60 g, it corresponds to a d_{O_2} of about 0.1 ml·day$^{-1} \cdot torr^{-1} \cdot (g_{egg})^{-1}$) even if $\dot{q}_{sh}$ is assumed to be zero. Conversely, even if $\dot{q}_{sh}$ reaches 20%, the D_{O_2} has to be smaller than 10 $\mu l \cdot min^{-1} \cdot torr^{-1}$ (corresponding to a d_{O_2} of about 0.24 ml·day$^{-1} \cdot torr^{-1} \cdot (g_{egg})^{-1}$), because the end-capillary blood P_{O_2} calculated in the present analysis could not exceed the $P_{A_{O_2}}$. Similarly, the allantoic shunt could not exceed 20%. As the allantoic shunt increases, the D_{O_2} should increase to yield a given $\Delta P_{A_{O_2}} \cdot P_{a_{O_2}}$ (Fig. 5). As the D_{O_2} is increased, however, the calculated $P_{c_{O_2}}$ exceeds $P_{A_{O_2}}$, limiting the upper $\dot{q}_{sh}$ values in the analysis. Suppose the allantoic shunt is 10%, the D_{O_2} of control eggs ranges from 4.6 to 8.3 $\mu l \cdot min^{-1} \cdot torr^{-1}$ (corresponding to a d_{O_2} of about 0.1 to 0.22 ml·day$^{-1} \cdot torr^{-1} \cdot (g_{egg})^{-1}$) with an average of 6.1 ± 1.0 (SD) (N = 27) $\mu l \cdot min^{-1} \cdot torr^{-1}$. The average D_{O_2} for $\dot{q}_{sh}$ of 0, 5, 15 and 20% is 5.3 ± 0.8 (N = 27), 5.6 ± 0.9 (N = 27), 6.8 ± 1.2 (N = 22) and 7.4 ± 1.1 (N = 14), respectively.

Comparison between the variables shown in Figs. 4 and 5 shows that changes in $\Delta P_{A_{O_2}} \cdot P_{a_{O_2}}$ with g_{O_2} make a mirror image of those in the diffusing capacity. It may suggest that when the shell conductance is increased by removing in part the shell, the diffusing capacity of the inner barrier (the inner shell membrane, the chorioallantoic membrane and the chemical reaction with hemoglobin) is decreased, contributing large $\Delta P_{A_{O_2}} \cdot P_{a_{O_2}}$ at high g_{O_2}. Suppose the allantoic shunt changes insignificantly with altered shell conductance, changes in the diffusing capacity with g_{O_2}
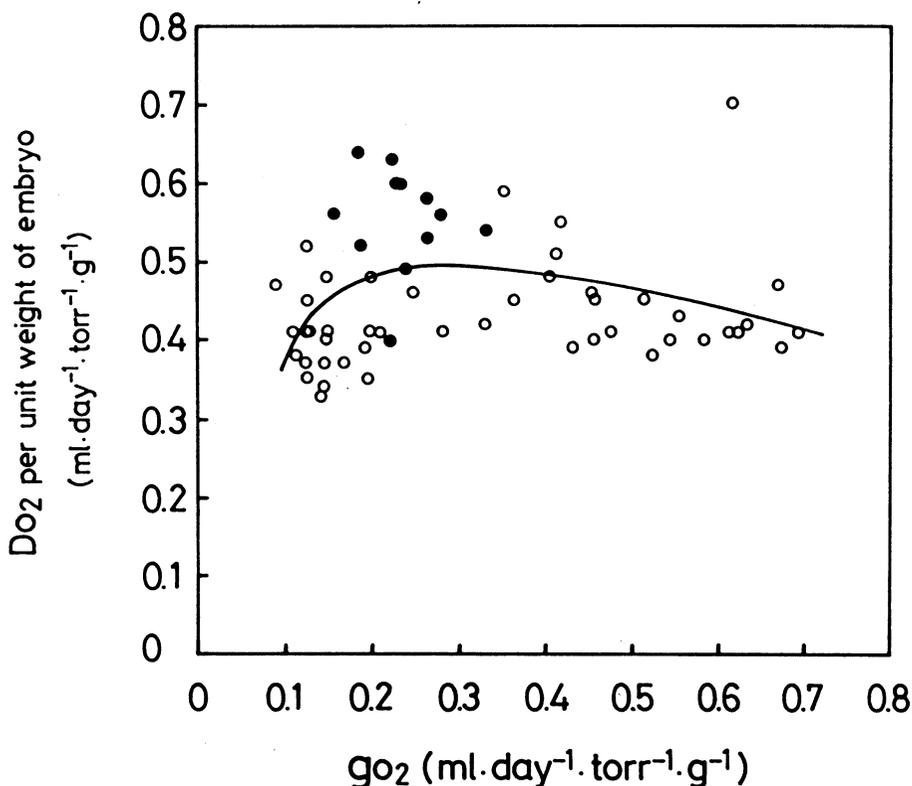
Fig. 7. Diffusing capacity per unit weight of embryo (mass$_{(embryo)}$-specific diffusing capacity) plotted for each shell conductance. The solid curve shows the regression equation.

seems to be paralleled with those in the wet weight of embryos. But, the decrease in the diffusing capacity is large compared with those in embryo weights, the D_{O_2} per g embryo (mass$_{(embryo)}$-specific diffusing capacity, $D_{O_2\ (specific)}$)being decreasing with increasing g_{O_2}. The $D_{O_2\ (specific)}$ also decreases at lowered g_{O_2}. In Fig. 7 are plotted the $D_{O_2(specific)}$ for individual values of g_{O_2} when the allantoic shunt is assumed to be 10%. The best-fit regression curve for relation between $D_{O_2(specific)}$ and g_{O_2} is expressed by

$$D_{O_2\ (specific)} = 0.69 - 0.027\ g_{O_2}^{-1} - 0.34\ g_{O_2}$$

where the hyperbolic and linear parameters are significant (F = 7.46, P < 0.01 and F = 6.13, P < 0.05, respectively). The $D_{O_2\ (specific)}$ of control eggs (closed circles) is large compared with that of

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conductance-decreased and -increased eggs.

The $D_{O_2(specific)}$ is almost comparable to the diffusing capacity of human lungs expressed per unit weight of the body.　In the chorioallantoic gas exchanger, nevertheless, the end-capillary blood P_{O_2} fails to reach the air space P_{O_2} and the blood has to leave the chorioallantoic capillary without being fully oxygenated.　This may partially be attributed to the structure of the gas ex-changer.

For control eggs, supposing the allantoic shunt is 10%, the ratio of the allantoic diffusing capacity to the shell diffusive conductance is 0.60 ± 0.17 (average $\pm SD$, $N = 27$).　The resistance of the inner barrier to O_2 diffusion is about 1.7-fold that of the shell.　A similar ratio has been reported for resistances to CO diffusion in the hens egg incubated for 16 days (Bissonnette and Metcalfe, 1987).

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