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# Effects of long-term alteration of the shell conductance on the blood gases and hematological variables in chick embryos

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## Abstract

- (1) Water vapor conductance ( $g_{H_2O}$ ) of the shell was widely altered at the beginning of incubation, and blood gas and hematological variables were determined on day 16 of incubation.
- (2) Changes in blood gas tensions ( $Pa_{O_2}$  and  $Pa_{CO_2}$ ), hematocrit and mean corpuscular indices were expressed by quadratic regression equation of inverse  $g_{H_2O}$ .
- (3) In connection with lowered  $g_{H_2O}$ , the reduced  $Pa_{O_2}$  might contribute to increases in both cell count and macrocytes and concurrent hypercapnia, increase mean cell volume (MCV).
- (4) The marked increase in MCV along with small decrease in mean corpuscular hemoglobin at lowered  $g_{H_2O}$  accounted for prominent decrease in mean cell hemoglobin concentration. The whole blood hemoglobin concentration was however maintained high by increased cell count.

## 1. Introduction

The gas exchange of incubated avian eggs takes place by diffusion across the eggshell. The diffusive conductance of the shell ( $G$ ), which is determined by effective pore area ( $A_p$ ), pore length ( $L_p$ ), gas diffusion coefficient ( $D$ ) and gas capacitance coefficient ( $\beta$ ) as  $G = (A_p/L_p) \cdot D \cdot \beta$ , is equivalent to the convective conductance which comprises pulmonary ventilation times capacitance coefficient in animals having lungs (Rahn *et al.*, 1971; Rahn and Paganelli, 1982). The previous study (Tazawa *et al.*, 1983) showed that in the diffusive transport system the hyperbolic relation also exists between naturally varying eggshell conductance and blood  $P_{CO_2}$  as dose between alveolar ventilation and alveolar or arterial  $P_{CO_2}$ . For these eggs selected from natural variation of shell conductance, it was also suggested that the arterialized blood  $P_{O_2}$  ( $Pa_{O_2}$ ) was hyperbolically diminishing with decreasing conductance and the hematocrit value tended to increase. Because the shell conductance is altered by varying  $A_p$  more widely than natural variation, the experiment was designed to investigate more closely the effects of widely altered shell conductance on blood gas properties and hematological variables (hematocrit value, erythrocyte count, blood hemoglobin concentration, and mean corpuscular indices) of chick embryos.

## 2. Materials and methods

Fertile chicken eggs were at first measured for length (L in cm) and maximum breadth diameter (B in cm) to estimate the initial egg mass (W in g) using equation,  $W = 0.5632 B^2 \cdot L$  (Romanoff and Romanoff, 1949). Then, the eggshell was coated with epoxy cement over the sharp end or removed along with outer shell membrane over the air cell at various sizes to provide wide range of shell conductance. The maximum coated areas extended over half end of the egg and the maximum holes over the air cell had diameter of 10-12 mm. These eggs along with intact ones (controls) were incubated at 38°C and about 55% relative humidity for 16 days. Eggs were turned automatically every 3 hours to maintain adequate gas exchange and development of embryos (Tazawa, 1980). On days 14-15 of incubation, the water vapor conductance ( $G_{H_2O}$  in  $mg \cdot day^{-1} \cdot torr^{-1}$ ) of individual eggs was determined using the "calibrated egg" technique as described previously (Tullett, 1981). For standardization, the water vapor conductance was expressed as specific water vapor conductance ( $g_{H_2O}$  in  $mg \cdot day^{-1} \cdot torr^{-1} \cdot g^{-1}$ ) by dividing by initial egg mass as mentioned in the previous studies (Tazawa *et al.*, 1983; Visschedijk *et al.*, 1985).

On day 16 of incubation, 0.4 ml blood was sampled with Hamilton syringe (No. 750), the dead space of which had been reduced to about 10  $\mu$ l by appropriately grinding down the tip of the plunger, from the allantoic vein or artery which was previously located by candling. Immediately after sampling, the blood was analyzed for  $Po_2$ ,  $Pco_2$  and pH with Instrumentation Laboratory blood gas analyzer (type 326 and 213), for hematocrit value (Hct in %) with Compur microcentrifuge (M1100) and for hemoglobin concentration (Hb in g%) and red blood cell count (RBC count in million  $\cdot mm^{-3}$ ) with Microcell counter (Sysmex CC-130 and AD-241, Tōa Medical Elect., Japan). Using cyanmethemoglobin method, the Hb was determined for supernatant solution after hemolyzed blood was centrifuged. Because blood  $Po_2$  reading was decreasing exponentially with lapse of time after injection of blood into the electrode chamber,  $Po_2$  was extrapolated from values recorded every minute up to 5 min of injection.  $Pco_2$  and pH were read 2 min after injection. The mean corpuscular indices (mean corpuscular volume; MCV in  $\mu^3$ , mean corpuscular hemoglobin; MCH in pg, and mean corpuscular hemoglobin concentration; MCHC in g%) were calculated as follows,

$$MCV = 10 \text{ Hct} / (\text{RBC count})$$

$$MCH = 10 \text{ Hb} / (\text{RBC count})$$

$$\text{and } MCHC = 100 \text{ Hb} / \text{Hct}$$

The relation between given variables was expressed by linear, quadratic or partial regression equation derived by the least square method. The significance of correlation between two variables

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was ascertained by Student t-test. The significance for quadratic parameter and individual parameters of the partial regression equation was examined by Fisher F-test.

### 3. Results

Two hundred nineteen eggs were subjected to determination of water vapor conductance, blood gas properties and hematological variables, in which one hundred eleven eggs had blood sampled from the allantoic vein and others (N=108), from the allantoic artery. The average mass of newly laid eggs calculated from length and breadth was  $64.5 \pm 2.5$  (SD) g (N=219). The water vapor conductance ( $G_{H_2O}$  in  $mg \cdot torr^{-1} \cdot day^{-1}$ ) of intact control eggs averaged out to  $16.7 \pm 3.5$  (SD, N=75), which corresponded to be specific water vapor conductance ( $g_{H_2O}$  in  $mg \cdot torr^{-1} \cdot day^{-1} \cdot g^{-1}$ ) of  $0.259 \pm 0.051$  (SD, N=75). For intact control eggs, the values of variables determined for blood from the allantoic vein and artery are tabulated in Table 1. Except blood gas properties and mean corpuscular volume (MCV), no variables were statistically different between vein and artery blood. MCV in the allantoic artery was significantly larger than that in the allantoic vein ( $P < 0.05$  by unpaired t-test).

Table 1. Variables determined for control eggs.

	No	Weight		Conductance		Po <sub>2</sub> (torr)	Pco <sub>2</sub> (torr)	pH	Hct (%)	RBC	Hb (g%)	MCV (μ <sup>3</sup> )	MCH (pg)	MCHC (g%)
		Egg (g)	Embryo (g)	G <sub>H<sub>2</sub>O</sub>	g <sub>H<sub>2</sub>O</sub>									
vein	39	63.9 ±2.4	17.5 ±2.2	16.4 ±3.5	0.254 ±0.047	67 ±8	29.6 ±6.2	7.53 ±0.06	32.0 ±3.4	2.45 ±0.26	9.0 ±1.1	131 ±7	36.6 ±2.2	27.9 ±1.2
artery	36	64.3 ±2.4	18.2 ±2.0	17.0 ±3.4	0.264 ±0.054	18.3 ±3.6	38.2 ±5.2	7.46 ±0.05	32.8 ±3.7	2.44 ±0.30	8.9 ±1.3	135 ±9	36.7 ±2.3	27.2 ±1.4
total	75	64.1 ±2.4	17.8 ±2.1	16.7 ±3.5	0.259 ±0.051				32.4 ±3.6	2.44 ±0.28	9.0 ±1.2		36.6 ±2.2	27.6 ±1.3

G<sub>H<sub>2</sub>O</sub> : water vapor conductance in  $mg \cdot day^{-1} \cdot torr^{-1}$

g<sub>H<sub>2</sub>O</sub> : specific water vapor conductance in  $mg \cdot day^{-1} \cdot torr^{-1} \cdot g^{-1}$

RBC : red blood cell count in  $millions \cdot mm^{-3}$ .

The water vapor conductance ( $G_{H_2O}$ ) which was artificially altered ranged from 6.6 to 63  $mg \cdot torr^{-1} \cdot day^{-1}$ , corresponding to the specific conductance ( $g_{H_2O}$ ) from 0.105 to 0.949  $mg \cdot torr^{-1} \cdot day^{-1} \cdot g^{-1}$ . Fig. 1 shows changes in blood Po<sub>2</sub> and Pco<sub>2</sub> with widely altered  $g_{H_2O}$ . Po<sub>2</sub> and Pco<sub>2</sub> of arterialized blood drawn from the allantoic vein (referred to as Pa<sub>O<sub>2</sub></sub> and Pa<sub>CO<sub>2</sub></sub>, respectively) are shown by closed circles and solid regression curves. The curves are expressed by

$$Pa_{O_2} = 68.3 + 1.54 g_{H_2O}^{-1} - 0.574 g_{H_2O}^{-2} \quad (N=111) \quad (1)$$

and  $Pa_{CO_2} = 12.2 + 5.23 g_{H_2O}^{-1} - 0.197 g_{H_2O}^{-2} \quad (N=111) \quad (2)$

where the quadratic parameters are significant at  $F = 45.99$  ( $P < 0.01$ ) and  $F = 20.28$  ( $P < 0.01$ ) for eqs. (1) and (2), respectively. For blood drawn from the allantoic artery,  $Po_2$  ( $Pv_{O_2}$ ) is shown by open circles and broken regression curve and  $Pco_2$  ( $Pv_{CO_2}$ ) is indicated by only broken regression curve. The best-fit curves are expressed by

$$Pv_{O_2} = 20.6 - 0.56 g_{H_2O}^{-1} \quad (N=108, r=0.248, P < 0.01) \quad (3)$$

and  $Pv_{CO_2} = 25.0 - 32.1 g_{H_2O}^{-1} \quad (N=108, r=0.790, P < 0.001) \quad (4)$

where the correlations between gas tensions and inverse  $g_{H_2O}$  are significant.

Changes in pH of arterialized blood with altered  $g_{H_2O}$  are presented in Fig. 2. Control eggs are indicated by closed circles. Open circles plotted in a lower range of

$g_{H_2O}$  show eggs whose respiratory areas were reduced by coating with epoxy cement (referred to as conductance-decreased eggs). Those in a higher range of  $g_{H_2O}$  indicate eggs whose shell was partially removed (referred to as conductance-increased eggs). The average values with SD are shown by rectangle with bars for groups control, conductance-decreased and conductance-increased eggs, respectively.

Fig. 3 shows hematocrit value (upper), erythrocyte count (middle) and hemoglobin concentration (lower panel) determined for 219 eggs. Values for the allantoic vein blood are plotted by closed circles and those for the allantoic artery blood, by open circles. The quadratic regression curve (solid curve in the upper panel) expressed by

$$Hct = 33.8 - 1.05 g_{H_2O}^{-1} + 0.199 g_{H_2O}^{-2} \quad (N=219) \quad (5)$$

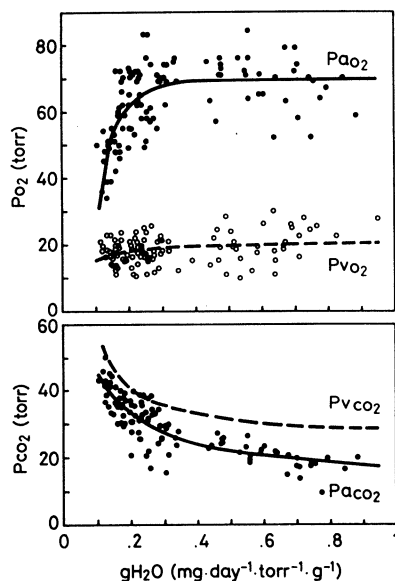


Fig. 1. Blood  $Po_2$  and  $Pco_2$  determined for 219 eggs with widely altered shell conductances. Gas tensions arterialized blood in the allantoic vein ( $Pa_{O_2}$  and  $Pa_{CO_2}$ ,  $N = 111$ ) are indicated by closed circles and solid regression curves. Those of the allantoic artery blood ( $Pv_{O_2}$  and  $Pv_{CO_2}$ ,  $N = 108$ ) are shown by open circles and broken regression curve or by only broken regression curve.

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fits well the relation between Hct and  $g_{H_2O}$ , where quadratic parameter is significant at  $F = 17.80$  ( $P < 0.01$ ). However, red blood cell count and hemoglobin concentration of blood are not significantly related to widely altered  $g_{H_2O}$ . Regression curves shown in the middle and lower panels are drawn from the quadratic equations of inverse  $g_{H_2O}$  whose first and second power parameters are not significant.

The mean corpuscular indices are plotted in Fig. 4, where the allantoic vein and artery bloods are presented by closed and open circles, respectively.

The best-fit regression curves are expressed by

$$MCV = 134 - 2.1 g_{H_2O}^{-1} + 0.52 g_{H_2O}^{-2} \quad (N=219) \quad (6)$$

$$MCH = 37.0 - 0.06 g_{H_2O}^{-1} - 0.013 g_{H_2O}^{-2} \quad (N=219) \quad (7)$$

and  $MCHC = 27.9 + 0.26 g_{H_2O}^{-1} - 0.097 g_{H_2O}^{-2} \quad (N=219) \quad (8)$

where quadratic parameters are all significant;  $F = 38.99$  ( $P < 0.01$ ),  $10.40$  ( $P < 0.01$ ) and  $120.58$  ( $P < 0.01$ ) for MCV, MCH and MCHC equations, respectively.

## 4. Discussion

### Control values

Control eggs in the present study ( $N = 75$ , Table 1) have specific water vapor conductances which are similar to those determined previously for 395 eggs (Visschedijk *et al.*, 1985). The average values of arterialized blood gas tensions and pH reported previously for 16-day-old chick embryos (summarized by Olszowka *et al.*, 1987) range from 55 to 71 torr for  $P_{O_2}$ , from 25 to 43 torr for  $P_{CO_2}$  and from 7.46 to 7.61 for pH. There are wide variations in average values among different studies and even in individual values of the present determination. The average values of Hct, RBC count and Hb which were previously reported for 16-day-old embryos are in a range of about 27 to 35 %, 2 to 2.6 million  $\cdot$  mm<sup>-3</sup> and 8 to 10 g%, respectively (Yosphe-Purer *et al.*, 1953; Johnson, 1955; Rychter *et al.*, 1955; Macpherson and Deamer, 1964; Romanoff, 1967; Freeman and Misson, 1970; Temple and Metcalfe, 1970; Tazawa *et al.*, 1971; Lemez, 1972; Tazawa,

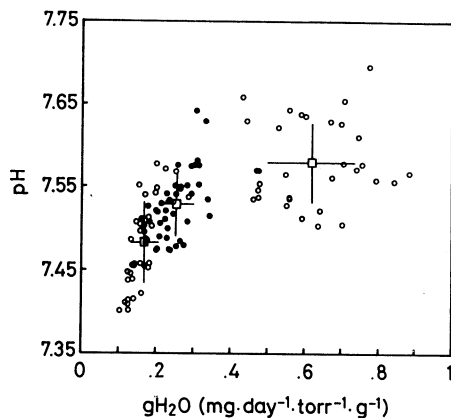


Fig. 2. pH of arterialized blood determined for 111 eggs with widely altered shell conductances. Intact (control) eggs are presented by closed circles.

1980). While these three variables are increasing with embryonic development, the mean corpuscular indices become unchanged during the last half of prenatal period, *i.e.*, MCV has been reported to be in a range of 110 to 190  $\mu^3$ ; MCH, 35 to 42 pg; and MCHC, 26 to 32 g% (O'Connor, 1952; Barnes and Jensen, 1959; Macpherson and Deamer, 1964; Romanoff, 1967; Ackerman, 1970; Freeman and Misson, 1970). The average values of these hematological variables determined for control eggs of the present study are all within the range of the previous reports.

*Effects of  $g_{H_2O}$  on blood respiratory properties*

In the previous study analyzing relations between naturally varying shell conductance and blood gas tensions (Tazawa *et al.*, 1983) the relation between  $g_{H_2O}$  and arterialized blood gas tensions was shown to be hyperbolic and simultaneously it was suggested that the hyperbolic relation was somewhat flattened due to metabolic rate which might be altered with  $g_{H_2O}$  values. On the basis of the

previous measurement of  $\dot{M}O_2$  consumption (Tullett and Deeming, 1982), Tullett and Burton (1985) assumed that the decrease in  $\dot{M}O_2$  with decreasing  $G_{H_2O}$  was hyperbolic and derived the quadratic equation of inverse  $G_{H_2O}$  for prediction of air cell  $PO_2$  ( $PA_{O_2}$ ). The best-fit regression equation for the relation between widely altered conductance and arterialized blood  $PO_2$  (and  $Pa_{CO_2}$ ) results in quadratic function of inverse of conductance in the present result. However, signs of the first and second power parameters of  $Pa_{O_2}$  equation (eq.(1)) do not agree with those of  $PA_{O_2}$  equation derived by Tullett and Burton (1985), suggesting the following. When eggs with increased conductance are included, the change in  $\dot{M}O_2$  with conductance may not be expressed by hyperbolic function. In this

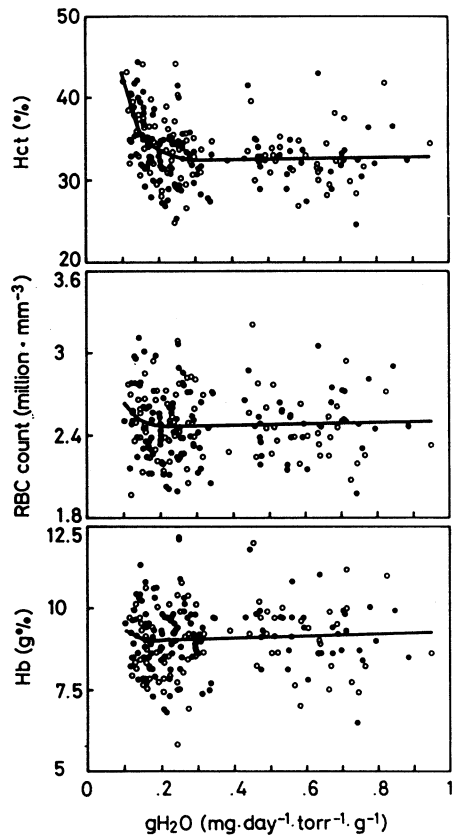


Fig. 3. Hematocrit value (Hct), erythrocyte count (RBC count) and whole blood hemoglobin concentration (Hb) determined for allantoic vein blood (closed circles, N=111) and allantoic artery blood (open circles, N=108) of eggs with widely altered conductances.



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connection, Visschedijk *et al.* (1985) suggested that the  $\dot{M}o_2$  decreased as the conductance increased above medium value.

The effect of widely altered conductance on  $\dot{M}o_2$  remains to be investigated. In addition, for the late chick embryos, it has been known that there is about 50-torr  $Po_2$  difference between air cell gas and arterialized blood which is attributed to the diffusing capacity of the chorioallantoic membrane and physiological shunt of the chorioallantoic circulation (allantoic shunt) (Piiper *et al.*, 1980). These factors might affect  $Po_2$  difference between air cell gas and arterialized blood at increased shell conductance. In fact, despite of increased conductance,  $Pa_{O_2}$  was not increased (Fig. 1). In a few eggs with increased  $g_{H_2O}$ ,  $Pa_{O_2}$  remained as low as the minimum value of the control, suggesting a decrease in diffusing capacity and/or increase in allantoic shunt as air cell  $Po_2$  increases above the control values.

The change in arterialized blood pH of control eggs is delimited within a range of about 0.1 unit except 2 eggs having high  $g_{H_2O}$  (Fig. 2). For these control eggs, the slope of relation between  $[HCO_3^-]$  and pH obtained by plotting individual values on pH- $[HCO_3^-]$  diagram is  $30.3 \text{ mM} \cdot \text{pH}^{-1}$ , while *in vitro* buffer lines have average slope of  $14.7 \text{ mM} \cdot \text{pH}^{-1}$  on day 16 of incubation (Tazawa, 1981). For a control range of  $g_{H_2O}$  except a few cases of high or low value, non-respiratory regulation of pH takes place as indicated previously (Tazawa *et al.*, 1983). Below and above this range of  $g_{H_2O}$ , pH becomes widely scattered. Particularly, pH decreases precipitously with decreasing  $g_{H_2O}$  in conductance-decreased eggs. Plasma  $[HCO_3^-]$  is not increased so large as expected from decreases in pH when  $g_{H_2O}$  is decreased below control.

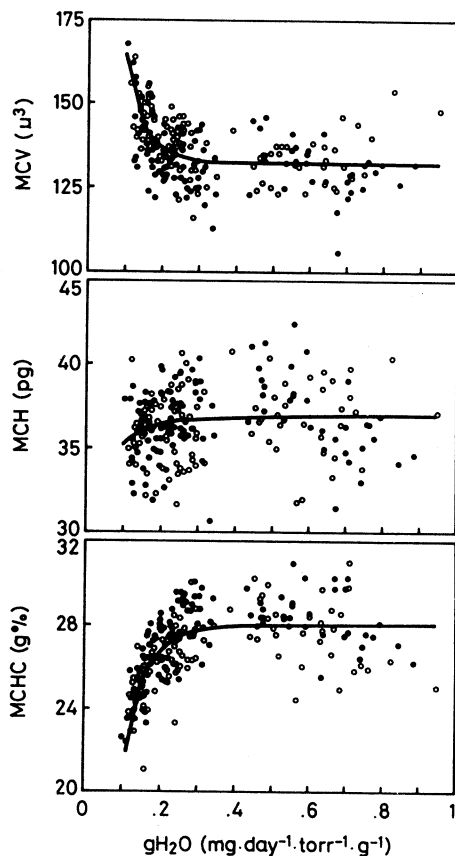


Fig. 4. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) of the same eggs as for Fig. 3.

*Effects of  $g_{H_2O}$  on hematological variables*

For eggs with naturally varying shell conductance, it was reported that Hct increased with decreasing  $g_{H_2O}$  (Tazawa *et al.*, 1983). The correlation was significant for 18-day-old embryos, but insignificant for 16-day-old embryos. The narrow range of  $g_{H_2O}$  based upon natural variation might partially account for insignificant correlation in 16-day-old embryos of previous study. The present result including eggs whose conductances were artificially altered shows that despite of wide scattering, Hct increases significantly with decreasing  $g_{H_2O}$  (Fig. 3). For a range of  $g_{H_2O}$  increasing beyond controls, however, Hct remains unchanged. The regression curve is expressed well by quadratic function of inverse  $g_{H_2O}$  as it is between  $Pa_{O_2}$  and  $g_{H_2O}$ . Not only Hct, but also RBC count and Hb which are insignificantly related to  $g_{H_2O}$  are correlated to  $Pa_{O_2}$  (Fig. 5). The correlation is expressed by the linear regression equation as follows,

$$Hct = 47.5 - 0.22 Pa_{O_2} \quad (N=111, r=0.621, P<0.001) \quad (15)$$

$$RBC = 2.99 - 0.008 Pa_{O_2} \quad (N=111, r=0.358, P<0.001) \quad (16)$$

and  $Hb = 10.4 - 0.019 Pa_{O_2} \quad (N=111, r=0.218, P<0.001) \quad (17)$

Changes in mean corpuscular indices are also expressed by quadratic equation of inverse  $g_{H_2O}$  (Fig. 4); below control  $g_{H_2O}$  they significantly decrease or increase with decreasing  $g_{H_2O}$ , but above the control they change little with increasing  $g_{H_2O}$  as does little the change in  $Pa_{O_2}$  with  $g_{H_2O}$ . The changes in these indices with  $g_{H_2O}$  are also mediated by  $Pa_{O_2}$  as expressed by following regression equations,

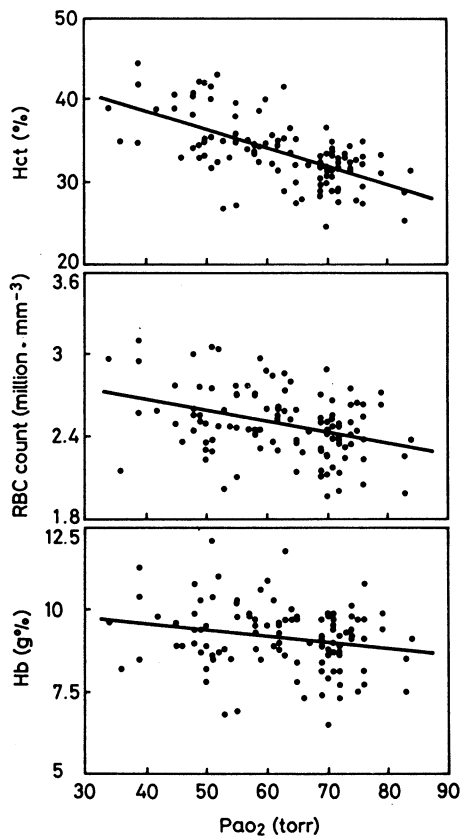


Fig. 5. Hematocrit value (Hct), erythrocyte count (RBC count) and whole blood hemoglobin concentration (Hb) plotted for individual allantoic vein blood  $PO_2$ s ( $Pa_{O_2}$ ).

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$$MCV = 163 - 0.46 Pa_{O_2} \quad (18)$$

(N=111, r=0.528, P<0.001)

$$MCH = 34.2 + 0.038 Pa_{O_2} \quad (19)$$

(N=111, r=0.200, P<0.05)

and  $MCHC = 20.1 + 0.115 Pa_{O_2} \quad (20)$

(N=111, r=0.722, P<0.001)

Fig. 6 shows the relation between mean corpuscular indices and  $Pa_{O_2}$ . Simultaneously, MCV and MCHC are also highly related to  $Pa_{CO_2}$  as follows,

$$MCV = 118 + 0.57 Pa_{CO_2} \quad (21)$$

(N=111, r=0.488, P<0.001)

and  $MCHC = 31.4 - 0.138 Pa_{CO_2} \quad (22)$

(N=111, r=0.644, P<0.001)

In consequence, MCV and MCHC are significantly related to both  $Pa_{O_2}$  and  $Pa_{CO_2}$  as expressed by following partial regression equations,

$$MCV = 146 - 0.32 Pa_{O_2} + 0.27 Pa_{CO_2} \quad (23)$$

(N=111)

and  $MCHC = 23.7 + 0.085 Pa_{O_2} - 0.058 Pa_{CO_2} \quad (24)$

(N=111)

where  $Pa_{O_2}$  and  $Pa_{CO_2}$  parameters are significant at  $F=10.57$  ( $P<0.01$ ) and  $F=4.27$  ( $P<0.05$ ) for eq. (23) and at  $F=35.23$  ( $P<0.01$ ) and  $F=9.19$  ( $P<0.01$ ) for eq. (24), respectively. Fig. 7 illustrates the partial regression equation (eq.(23)) and individually measured MCV plotted against  $Pa_{CO_2}$  and  $Pa_{O_2}$  on the three dimensional coordinates. The plane comprising 4 coordinate-points, (10,30,139)-(50,30,150)-(50,90,131)-(30,90,125), is expressed by eq. (23). The MCV measured, which is larger than the value calculated from eq. (23), is shown by closed circle and the difference from the plane is indicated by solid line. The open circle shows measured MCV which is

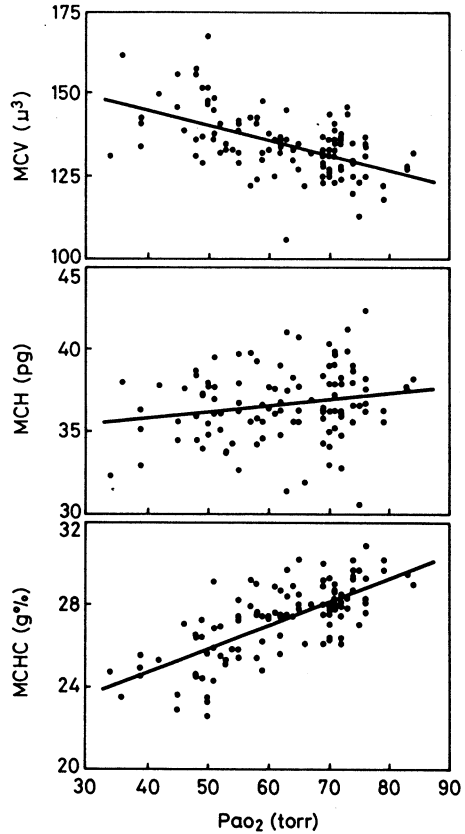


Fig. 6. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) plotted for individual  $Pa_{O_2}$ s.

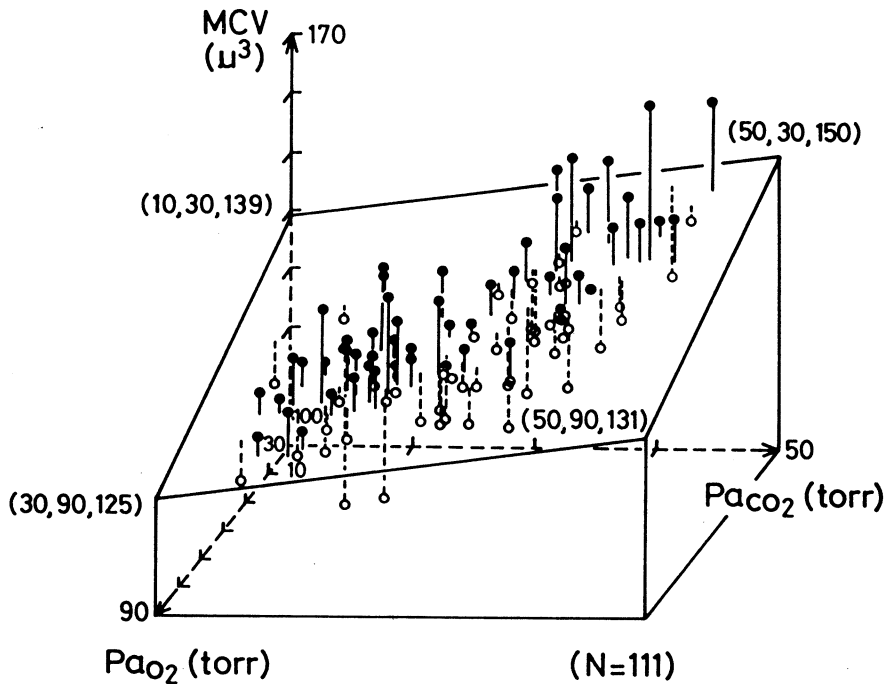


Fig. 7. Three dimensional presentation of mean corpuscular volume (MCV) in relation to arterial-ized blood  $P_{O_2}$  ( $P_{aO_2}$ ) and  $P_{CO_2}$  ( $P_{aCO_2}$ ).

smaller than the value predicted from eq. (23), and the broken line indicates difference from the plane. The mean corpuscular volume is increasing with hypoxia and concurrent hypercapnia and correspondingly the mean corpuscular hemoglobin concentration is decreasing.

Effects of ambient hypoxia on the erythrocytes of chick embryos have been reported to be different results. In embryos older than 16 days of incubation the continuous exposure to 15 %  $O_2$  exerted a stimulatory effect on Hct and Hb which exceeded those of the controls, but neither MCV nor MCH were affected (Jalavisto *et al.*, 1965). The results suggest that 15 %  $O_2$  induces erythropoiesis without discharging macrocytic precursors into the circulating blood. On the other hand, a 1-day exposure to 13 %  $O_2$  caused an increase in not only Hct and Hb, but also erythrocytic volume in embryos analyzed on days 15 and 17 of incubation (Ackerman, 1970). Based upon inspection of erythrocyte stage, Ackerman suggested that the enlarged MCV was due to a large number of circulating precursor stages.

The MCV which enlarged with decreasing  $g_{H_2O}$  in the present experiment might also be attrib-

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uted in part to circulating macrocytic precursors as a result of stimulatory effects of hypoxia on erythropoiesis. In addition, impediment of gas exchange due to decrease in  $g_{H_2O}$  causes an accumulation of  $CO_2$  in the egg, which increases intra-erythrocytic  $[HCO_3^-]$  causing enlargement of cell volume due to inward shift of water. The effect due to increased  $CO_2$  on erythrocyte volume may also account for enlarged volume of the allantoic artery erythrocyte of control eggs. The enlargement of erythrocytes due to increased  $CO_2$  however affects neither Hct nor cell hemoglobin concentration in control eggs (Table 1).

The enlarged MCV at decreasing  $g_{H_2O}$ , which may be attributed to both hypoxia and hypercapnia, on the other hand, contributes to the increase in Hct. The increase in Hct with decreasing  $g_{H_2O}$  is simultaneously attributed to erythrocyte count which is increased by hypoxia as a result of stimulatory effect on erythropoiesis. The Hct is highly is related to MCV and RBC count as expressed by following partial regression equation,

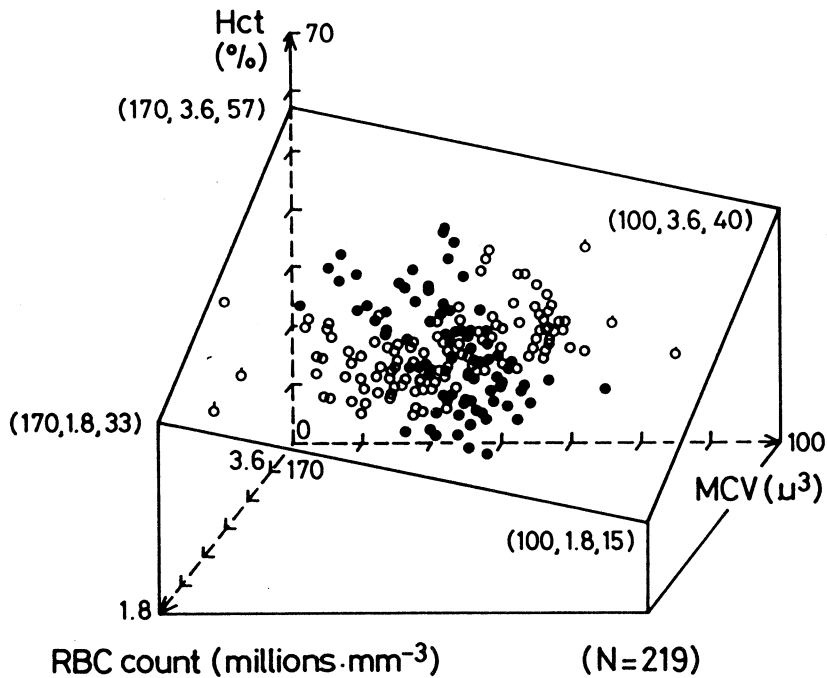


Fig. 8. Three dimensional presentation of hematocrit value (Hct) in relation to mean corpuscular volume (MCV) and erythrocyte count (RBC count).

$$Hct = -34.1 + 0.25 MCV + 13.65 RBC \quad (N=219) \quad (25)$$

where MCV and RBC count parameters are significant at  $F=17126$  and  $F=34661$ , respectively. Fig. 8 shows the Hct plotted on the 3 dimensional coordinates as a function of MCV and RBC number. The plane enclosed by (170,3.6,57)-(100,3.6,40)-(100,1.8,15)-(170,1.8,33) represents partial regression equation (eq.(25)). The Hct which is larger than the value calculated from eq. (25) is indicated by closed circle. The Hct which does not reach the average is shown by open circle. The deviation from this plane is indicated by bar and almost all the Hct value is located close to the plane. The plot indicates that while there is no correlation between RBC count and cell volume, each of them contributes to an increase in Hct.

Effects of possible dilution or concentration of the plasma volume caused by changing  $g_{H_2O}$  are not evident for changes in hematological variables.

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