Advances in Respiratory Physiology and Controls

Edited by

Masaji MOCHIZUKI

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ADVANCES IN RESPIRATORY PHYSIOLOGY AND CONTROLS

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Masaji MOCHIZUKI

Director of the Research Institute of
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Research Institute of Applied Electricity
Hokkaido University
Sapporo, Japan
1972

PREFACE

Recently, respiratory physiology and control studies have developed to be one of the main themes which are intensively investigated in the Research Divisions related to biomedical engineering in our Institute. The present volume of our Monograph Series has been planned in hopes to make valuable contributions to physiological knowledge and also to promote wide-spread cooperation among workers interested in these fields.

The first article is written by Dr. H. Tazawa, who devised a new microtechnique for O_2 and CO_2 analyses in blood three years ago in the Department of Biomedical Engineering, University of Virginia, U.S.A. Since then he has devoted himself to the study on gas exchange in the chicken embryo. His paper presented here contains the results of gas analyses of embryonic blood, which are relevant in the interpretation of the relationships between and among the diffusion and perfusion rates around the chorioallantoic capillaries and the metabolic rate.

The second two papers are presented by Dr. Y. Miyamoto. He commenced his studies on morphological observation of the red blood cell flowing through the capillaries in the Physiological Institute of Hanover, Germany five years ago in colse collaboration with Dr. W. Moll. The two articles show geometrical configurations of the red cell in the pulmonary and mesenteric capillaries. These data are of great importance in discussions of the reaction rate of the red cell with O_2 and CO in the lung.

The third article written by Dr. Miyamoto's team deals with the cause of phase difference between the airflow and the alveolar pressure observed in a body-plethysmograph. The relationship between the phase difference and airway resistance is mainly explained by a principle that the pulmonary airway is constructed by a series of resistances and compliances.

The fourth paper is written by Dr. H. Sugawara and his associates. Dr. Sugawara visited the laboratory of Dr. Wildevuur, State University of Groningen, The Netherlands in 1971, where he studied experimental lung transplantation. The article presented reveals the effect of denervation on the respiratory control. He clearly demonstrated that the ventilatory response to hypercapnia in denerved dogs is secured in a normal range by an increase of tidal volume which compensates for no response in respiratory frequency.

The fifth article is written by Dr. T. Mikami and his group, who are occupied in elaborating a respirator controlled by the metabolic rate of patients. Emphasis is placed on the following points: 1) The CO_2 output is used as an input signal for the control, 2) the arterial CO_2 tension is maintained constant and 3) the device is minimized for easy transportation by using a number of IC circuits for computation.

The final three articles presented by the editor's group treat fundamental problems of the pulmonary diffusing capacity. Quantitative values are shown on the combination rate of CO with the oxygenated red cell not only *in vitro*, but also in the pulmonary capillary, in addition, quantitative values on the contact time of the red cell with the alveolar air are also given. The characteristic feature of their treatment is as follow: A series of overall combination rates is calculated on a cylindrical red cell model covered with a uniform diffusion barrier under various diffusion conditions and the rate constant in the pulmonary capillary is estimated by checking the proportionality between the calculated rates and the pulmonary diffusing capacity.

The editor wishes to express his cordial thanks to the authors for their contributions.

Editor

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GAS EXCHANGE IN CHICKEN EMBRYO

HIROSHI TAZAWA

Abstract. To investigate chicken embryonic respiration, blood P_{O_2}, P_{CO_2}, pH, Hb, plasma $[HCO_3^-]$, total-CO_2 content and base excess were determined in a single blood sample drawn either from the chorioallantoic vein or artery of normally incubated embryos. We also studied the effects of the respiratory area, the diffusing rate and the incubative temperature on the gas exchange of embryonic blood.

The normally incubated embryos initially encountered respiratory alkalosis with metabolic acidosis and their blood P_{O_2} remained high in the middle stage of development. Then, the acid-base balance approached adult hen values with the lapse of time and P_{O_2} severely diminished as a result of rather constant diffusing capacity of the shell and the increasing O_2 uptake. The chorioallantoic membrane component of diffusing capacity predominantly increased during development, while the value per kg embryo tended to decrease and showed comparable values with other mammalian fetuses.

The decrease in the respiratory area resulted in the increase in the tension differences; in other words, the embryo was forced to meet severe hypoxia and its acid-base status inclined to respiratory acidosis. On the other hand, the increase in the diffusing rate through the air space diminished the CO_2 pressure difference and tended to respiratory alkalosis. Since the change in the environmental temperature around the eggs mainly affects the metabolic rate and permeability, the low temperature exposure resulted in notable respiratory alkalosis as observed in poikilothermic animals.

Introduction

Gas exchange in animals takes place either by convection or diffusion between the environment and blood in the gas exchange medium. When we think of respiration accomplished by vertebrates, four respiratory organs are mainly cited; namely, the lung, the gills, the placenta and the chorioallantois. The former two functionally come under convective gas exchange and the latter two come under diffusion gas exchange. RAHN, WANGENSTEEN and FARHI (1971) presented basic gas exchange equations for the four kinds of respiration. These recent trends toward the comparative physiology prompted us to provide data indicating the gas exchange in the chorioallantoic membrane of the avian embryo.

From a viewpoint of the type of the gas exchange (diffusion gas exchange), the respiratory system of the avian embryos may be considered as a simplified model of the mammalian fetus, because the mother can be completely replaced by an egg incubator. The investigation of the gas exchange in the avian embryo, therefore, may be useful for studies on the respiration of the mammalian fetus. Nevertheless, only a few data are available to the present concerning the gas exchange in avian embryonic blood (BARTELS, HILLER and REINHARDT, 1966; DAWES and SIMKISS, 1969; FREEMAN and MISSON, 1970 and ERASMUS, HOWELL and RAHN, 1970/71). We also studied the gas exchange properties of chicken embryonic blood (TAZAWA, 1970; TAZAWA, 1971; TAZAWA, MIKAMI and YOSHIMOTO, 1971 a and b). However, additional data are required for investigating the avian embryonic respiration and to further comparative studies on the blood gas exchange.

In diffusion gas exchange, the amount of gases diffusing through the respiratory surface per unit time is governed by the permeability, the respiratory area and the tension difference. These respiratory factors can readily be altered in eggs to determine their respective effects on the gas exchange, whereas some of the factors could not be controlled in the mammalian fetus. TEMPLE and METCALFE (1970) studied the effects of increased incubator oxygen tension on the capillary development in the chorioallantois. In the present report, we changed the respiratory area, the diffusing rate through the air space and the environmental temperature around the eggs and studied their respective effects on the blood gas exchange together with the respiratory properties of normally incubated embryo blood.

Materials and methods

BLOOD SAMPLING FROM THE CHORIOALLANTOIC BLOOD VESSELS

The gas exchange medium in the avian embryo is a chorioallantoic vascular plexus which corresponds to the fetal placenta in the mammalian uterus. The chorioallantois which is primarily a storage space of the waste products incident to metabolism is well vascularized and stretches widely under the inner surface of the shell with the progress of development. After the middle stage of incubation, therefore, the chorioallantoic membrane appears to envelop the entire embryo and thus the gas exchange completely depends on the chorioallantoic vascular capillaries. The chorioallantoic artery carries mixed venous blood as well as the waste products from the embryo to the chorioallantois. Then, the blood is well oxygenated when it passes through the capillaries, and is collected at the chorioallantoic vein and returns to the embryo. Since the blood vessels can be located from the outside of the shell by candling the egg, the site for blood withdrawal was previously marked on the egg. The shell was cracked with a needle and removed carefully with a pair of small forceps, and then the chorioallantoic membrane was torn along the allantoic vein or artery so as not to break the small vessels on its surface. The blood vessel selected for withdrawal was raised slightly with the forceps and the blood stream was occluded. Then, a fine needle attached to a syringe was immediately inserted into the vessel and blood was gradually withdrawn. The removed area of the shell was usually limited to less than 1.5 cm^2 and the time required for blood withdrawal was within 2 to 3 minutes after removing the egg from the incubator. The temperaure around the egg was kept at the incubative temperature in order to avoid possible thermal effects on the embryonic gas exchange.

BOOLD GAS ANALYSES

Whole blood O_2 content, O_2 capacity, P_{O_2}, P_{CO_2}, pH and hematocrit value (Hct) were measured. In addition, the weight of the individual embryo was determined with a precision balance after blood gas analysis.

The electrode method was used for measurement of O_2 content and O_2 capacity, which requires only a blood volume of 10 μl (TAZAWA, 1970). Blood P_{O_2}, P_{CO_2}, pH and Hct were simultaneously measured in single blood sample withdrawn from each of the embryos. A Radiometer Blood Micro System (type BMS 3), which requires about 100 μl of blood, was used for the simultaneous determination of P_{O_2}, P_{CO_2} and pH. The temperature of water bath was kept at the egg temperature during analysis. Hct was measured with a microhematocrit centrifuge which required approximately 50 μl of blood. Hemoglobin concentration (Hb) was calculated using a regression equation of Hct to O_2 capacity which was divided by 1.34. Blood P_{CO_2} and pH and calculated Hb served to obtain plasma $[HCO_3^-]$, total-CO_2 content and base excess using the Siggaard-Andersen alignment nomogram (SIGGAARD-ANDERSEN, 1963).

ALTERING THE FACTORS GOVERNING EMBRYONIC RESPIRATION

Since the surface area of an egg shell is responsible for the respiratory area after the chorioallantoic membrane envelops the entire embryo, the diminution of the respiratory area can easily be accomplished by coating the egg shell with an impermeable substance. In this experiment, we used epoxy cement applying a thin layer on the shell.

In the avian eggs, the diffusing rate can be changed by making a small window through the shell over the air space. For the purpose of increasing the diffusing rate, therefore, we opened an air space by drilling the shell.

The environmental or incubative temperature is an important factor for embryonic development and it also controls the permeability. Since embryonic development is directly concerned with the metabolic rate, we changed the temperature to study its effect on the blood gas exchange. Two means of changing the environmental temperature were used. One was to expose the eggs abruptly either to low or high temperature for a given period prior to blood withdrawal. In this case, the eggs were normally incubated at 38°C immediately prior to the exposure. The other method was to change the incubative temperature from the beginning of incubation. For this purpose, the eggs were incubated at 35°C throughout the entire incubating period.

Results and discussions

GAS EXCHANGE PROPERTIES OF NORMALLY INCUBATED EGGS

Since the temperature zone of survival at the time of hatching is limited to a range of 35°C to 40°C, 37.5°C or 38°C was considered to be optimal for development. Thus, the eggs incubated at 38°C were ragarded as normally incubated eggs. Therefore, the data here were obtained from eggs incubated at 38°C. Table 1 shows P_{O_2}, P_{CO_2}, pH, [HCO_3^-], total-CO_2 content and base excess of arterialized blood in the chorioallantoic vein (N=50 for each age) and of mixed venous blood in the chorioallantoic artery (N=20). The data presented here were accumulated from our four previous experiments. At the beginning of incubation, gas tensions in the eggs are considered nearly

TABLE 1

Blood gas properties of arterialzed blood (N=50) and mixed venous blood (N=20)

Age (days)		P_{O_2} (mmHg)	P_{CO_2} (mmHg)	pH	HCO_3^- (meq/l)	CO_2 content (mM/l)	B. E. (meq/l)
10	Arterialized blood	82.1 ± 4.9	11.4 ± 2.0	7.640 ± 0.046	12.3 ± 1.4	12.6 ± 1.4	− 8.1 ± 1.5
	Mixed venous blood	30.9 ± 11.5	17.6 ± 1.9	7.448 ± 0.030	12.0 ± 1.2	12.7 ± 1.2	− 10.8 ± 1.4
12	Arterialized blood	80.3 ± 3.3	15.2 ± 2.4	7.618 ± 0.038	15.5 ± 1.8	15.9 ± 1.8	− 5.0 ± 1.7
	Mixed venous blood	30.9 ± 10.2	21.0 ± 2.7	7.428 ± 0.055	15.0 ± 1.3	15.8 ± 1.3	− 8.2 ± 1.9
14	Arterialized blood	74.7 ± 5.0	23.0 ± 3.6	7.526 ± 0.045	18.8 ± 1.9	19.4 ± 1.9	− 3.0 ± 1.7
	Mixed venous blood	23.4 ± 9.3	35.0 ± 5.2	7.337 ± 0.046	18.4 ± 1.8	19.4 ± 1.9	− 6.4 ± 1.8
16	Arterialized blood	64.8 ± 6.8	31.0 ± 4.4	7.479 ± 0.045	22.9 ± 2.2	23.8 ± 2.3	− 0.1 ± 2.0
	Mixed venous blood	20.8 ± 6.1	43.6 ± 4.3	7.327 ± 0.056	22.5 ± 2.0	23.8 ± 2.1	− 3.0 ± 2.6
18	Arterialized blood	58.6 ± 7.7	33.7 ± 5.9	7.454 ± 0.072	23.3 ± 2.6	24.3 ± 2.7	− 0.1 ± 2.8
	Mixed venous blood	20.6 ± 4.9	45.2 ± 8.7	7.318 ± 0.055	22.6 ± 3.4	23.9 ± 3.5	− 3.1 ± 3.2

Values are means ± standard deviation. B. E.: Base excess.

equal to the values of atmosphere because of the negligibly small metabolic rate. Then, the growth of the embryo requires more O_2 from the ambient air and consequently a need to eliminate the increasing CO_2 product from the egg arises. Since a significant diffusion resistance exists in the egg shell and shell membranes (WANGENSTEEN, WILSON and RAHN, 1970/71 and KUTCHAI and STEEN, 1971), the increasing O_2 uptake and CO_2 production during development play a role to increase the tension difference. As a result, blood P_{O_2} decreases and P_{CO_2} increases with the progress of development. The arterialized blood P_{O_2} which shows 82 mm Hg on the 10th day of incubation further decreases its value to less than 60 mm Hg on the 18th day and P_{CO_2} increases from 11 mm Hg to 34 mm Hg. The comparatively low P_{CO_2} is accompanied with a high pH value (7.64 on the 10th day) which gradually decreases to the level of adult hen arterial blood (7.45 on the 18th day). Plasma $[HCO_3^-]$ and total-CO_2 content show low values concomitant with low P_{CO_2} and high pH, and notable base deficit was observed at the middle stage of incubation. Therefore, the acid-base status of embryonic blood is primarily in respiratory alkalosis with metabolic acidosis and approaches the adult hen level with the lapse of time. Before the middle stage of incubation, embryonic respiration takes place with comparative ease and arterialized blood P_{O_2} shows high values comparable with arterial blood P_{O_2} of the adult hen. Then, with the lapse of time, the embryo is forced to meet dyspneic respiration as a result of a rather constant diffusion resistance of the shell and the increasing metabolic rate. At the latest stage of incubation, the arterialized blood P_{O_2} becomes much lower than the hen arterial P_{O_2} and the embryo is forced to tolerate severe hypoxia. On the other hand, O_2 content in arterialized blood steadily increases during develop-

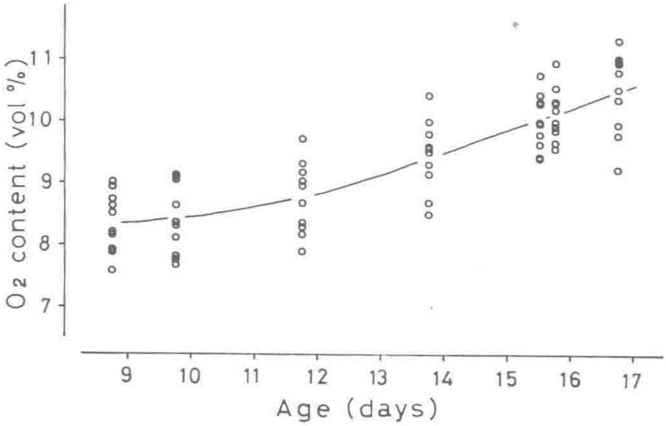

Fig. 1. Oxygen content in blood of the chorioallantoic vein. The increasing rate of O_2 content is linearly related to that of the embryonic weight.

ment incident to the increase in embryonic weight (Fig. 1) and O_2 capacity also increases from the value of 9.7 vol % on the 10th day to 12.2 vol % on the 18th day. The tendency in which O_2 capacity increases during incubation is favorable for transferring O_2 to the tissues at the time of hypoxia.

WANGENSTEEN and RAHN (1970/71) measured both P_{O_2} and P_{CO_2} in the air space during development. The air space P_{O_2} decreased from 137.5 mm Hg on the 10th day to 110 mm Hg on the 18th day and P_{CO_2} increased from 16 mm Hg to 39 mm Hg for the same period. Therefore, a difference of about 50 mm Hg in P_{O_2} between the air space and end-capillary blood appeared and there seemed to be no difference in P_{CO_2}. In other words, blood P_{O_2} was not equilibrated sufficiently to reach the air space P_{O_2}, while blood P_{CO_2} reached the air space P_{CO_2}. Therefore, it is obvious that the diffusing capacity for CO_2 in the chorioallantoic membrane is much higher than that for O_2. In avian embryos, the overall diffusing capacity ($D_{O_2,t}$) may be formulated as follows;

$$\frac{1}{D_{O_2,t}}=\frac{1}{D_{O_2,s}}+\frac{1}{D_{O_2,c}}+\frac{1}{\theta_e\cdot V_e}$$

where $D_{O_2,s}$ and $D_{O_2,c}$ are respectively the diffusing capacity of the shell with the membranes and of the chorioallantoic membrane, and θ_e and V_e respectively represent the oxygenation rate factor of hemoglobin and the capillary blood volume. Neglecting the oxygenation velocity of the red cell, we can further assume the overall diffusing capacity to be as follows;

$$\frac{1}{D_{O_2,t}}=\frac{1}{D_{O_2,s}}+\frac{1}{D_{O_2,e'}}$$

where only two diffusing capacities are concerned; one is for the shell with the shell membranes and another for the embryonic respiratory medium ($D_{O_2,e'}$). If the mean P_{O_2} gradient ($\Delta\bar{P}_{O_2}$) between the air and chorioallantoic capillary blood is given, the overall diffusing capacity for O_2 is calculated as $\dot{V}_{O_2}/\Delta\bar{P}_{O_2}$. The mean P_{O_2} gradient was obtained by using the Bohr's integral method on the assumption that HbO_2 dissociation curve does not change during development (Table 2). The HbO_2 dissociation curve used was that of the 17th day which was constructed by BARTELS, HILLER and REINHARDT (1966). The calculated overall diffusing capacity using the O_2 consumption values summarized by ROMANOFF (1967) is presented in Table 2. Similarly, $D_{O_2,s}$ and $D_{O_2,e'}$ were calculated by using the air space P_{O_2} measured by WANGENSTEEN and RAHN (1970/71) (Table 2). The results show that the egg shell component of diffusing capacity for O_2 ($D_{O_2,s}$) remains rather constant during development, while the chorioallantoic membrane component of diffusing capacity ($D_{O_2,e'}$) increases with time. For comparison with the diffusing capacities of the mammalian placentae compiled by BARTELS (1970),

TABLE 2

Tension differences and diffusing capacities

Embryonic age (days)	10	12	14	16	18
$\Delta\bar{P}_{O_2}$ (mm Hg)	101.9	103.1	108.6	110.6	111.9
ΔP_{O_2} (mm Hg) (air space to end-capillary blood)	55.4	51.0	49.8	47.1	51.3
$D_{O_2,t}$ ($ml \cdot min^{-1} \cdot mm\ Hg^{-1} \cdot 10^{-3}$)	0.61	1.18	1.86	2.50	2.77
$D_{O_2,s}$ ($ml \cdot min^{-1} \cdot mm\ Hg^{-1} \cdot 10^{-3}$)	5.36	6.85	8.26	7.44	7.94
$D_{O_2,e'}$ ($ml \cdot min^{-1} \cdot mm\ Hg^{-1} \cdot 10^{-3}$)	0.74	1.43	2.40	3.77	4.25
$D_{O_2,e'}$ ($ml \cdot min^{-1} \cdot mm\ Hg^{-1}$ per kg embryo)	0.36	0.33	0.29	0.27	0.22

we converted the $D_{O_2,e'}$ to the value per kilogram weight (Table 2). The values are well comparable with those of the mammalian placentae (see Table 5. 1 of BARTELS, 1970). Table 2 also shows P_{O_2} difference which appeared in the gas exchange medium (ΔP_{O_2} between the air space to end-capillary blood). This P_{O_2} difference was similarly observed in the mammalian placenta between the maternal artery and fetal umbilical vein.

EFFECTS OF THE REDUCED RESPIRATORY AREA AND THE INCREASED DIFFUSING RATE ON THE BLOOD GAS EXCHANGE

In order to determine these effects, we altered the shell either by coating or making a window, or both before starting incubation. The eggs in which the shell was modified were as follows. Group 1, the control eggs were incubated at 38°C without any modification. The shell in groups 2 and 3 were coated with epoxy cement to reduce the respiratory area either at the broad end (group 2) or narrow end (group 3). The coated area covered almost one-fourth of the total shell surface. In the eggs of groups 4 and 5, the shell over the air space was cut and a window of 3 to 4 mm in diameter was made in order to increase the diffusing rate. In addition to the window, the shells of the narrow end in the eggs of group 5 were coated with the epoxy cement to produce the combined effects of the decreased respiratory area and increased diffusing rate.

The results of blood P_{O_2}, P_{CO_2} and pH, and $[HCO_3^-]$ are shown in Figs. 2 a, b, c and d, respectively. The effect of blocking the gas exchange area on the blood gas properties was not significantly different between the broad end (group 2) and the narrow end (group 3); blood P_{O_2} diminished, P_{CO_2} increased markedly accompanying the decrease in pH, and $[HCO_3^-]$ increased.

On the other hand, P_{CO_2} was significantly diminished both in the two groups whose diffusing rate through the air space was increased (groups 4 and 5). However, no significant difference of P_{CO_2} between these two groups were observed, although the narrow end of one group (group 5) was coated with epoxy cement. While, blood P_{O_2} was not affected by the increase in the diffusing rate and was slightly decreased by the blocking of the respiratory area.

The effect of reducing the respiratory area resulted in severe hypoxia and predominant respiratory acidosis as compared with the control. The changes in blood gas tensions are explained by the decreased diffusing capacity provided the O_2 uptake and CO_2 production of the embryo remain normal.

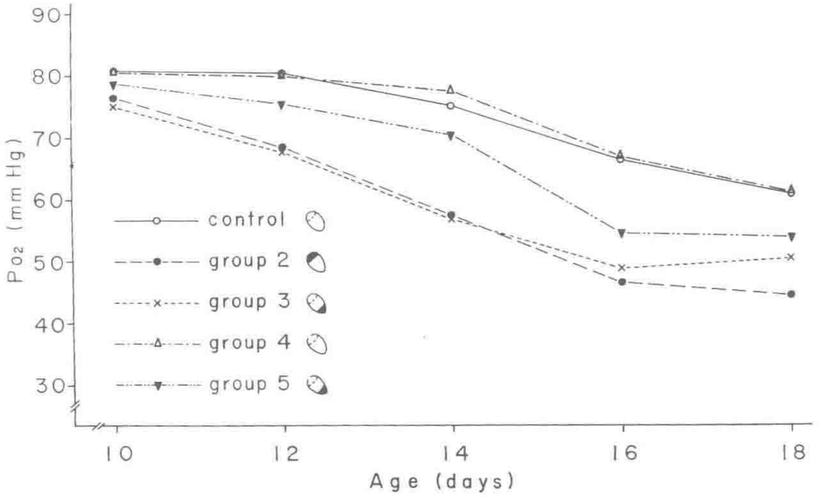

Fig. 2 a.

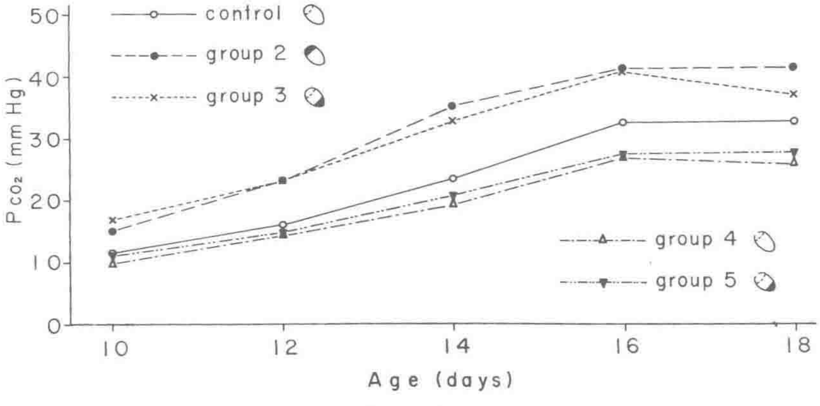

Fig. 2 b.

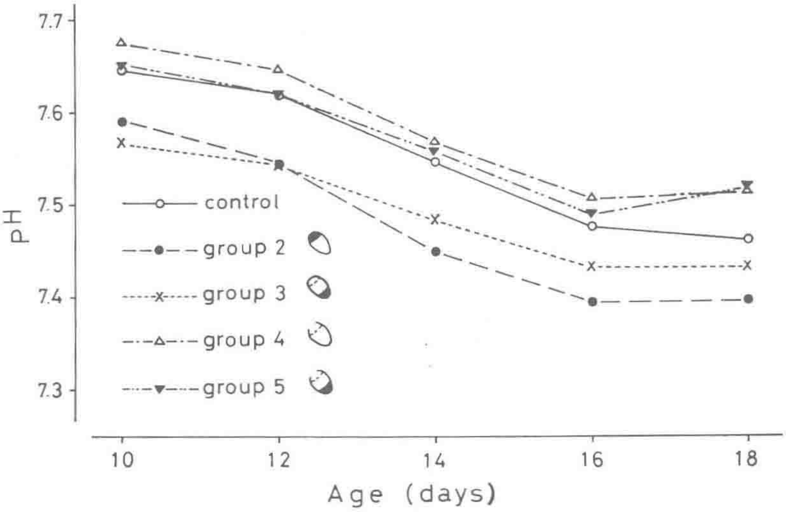

Fig. 2 c.

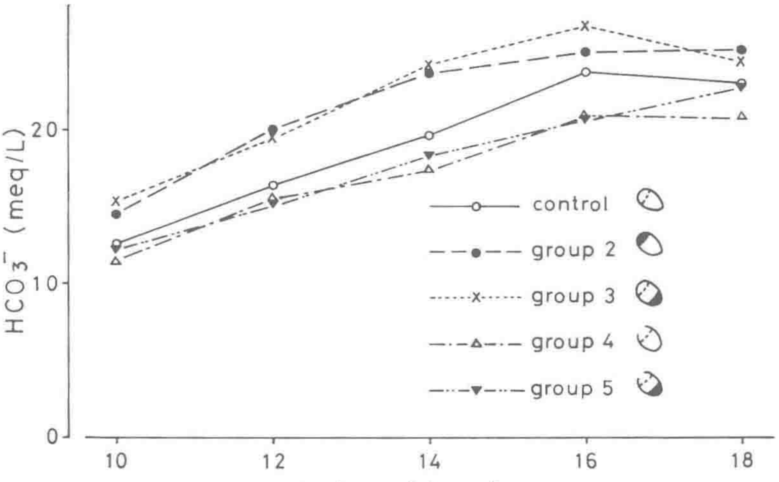

Fig. 2 d.

Fig. 2. Blood gas properties in the eggs incubated under various shell conditions which are depicted in the figure. The shaded area shows the shell surface coated by epoxy cement and the broken line, the area of the air space. Each value represents the average of ten determinations. a; P_{O_2}, b; P_{CO_2}, c; pH and d; [HCO_3^-].

Reduction of the diffusing area is considered equivalent to hypoventilation in animals whose respiration takes place by convection. The changes in blood gas properties, therefore, resemble the effect of hypoventilation in man.

The effect also had a tendency to increase Hct values and to retard embryonic development as compared with the control.

As a result of the increase in the diffusing rate, on the other hand, the embryo tended to respiratory alkalosis in comparison with the control. Blood P_{CO_2} did not reach the control level. On the contrary, the effect on blood P_{O_2} was much smaller than that on P_{CO_2}. This may depend on the facts that the respiratory area in the air space is small as compared with the total respiratory surface and the diffusing capacity for CO_2 is much higher than that for O_2. The increase in the diffusing rate tended to promote embryonic development.

TABLE 3

Hatchability

	number of eggs	number of embryos died before pipping	number of embryos died after pipping	number of chickens
group 1	16	0	1	15
group 2	18	3	5	10
group 3	14	2	4	8
group 4	18	1	1	16
group 5	14	4	3	7

The hatchabilities of these groups are shown in Table 3. Most of the embryos both in the control and group 4 hatched and no signigificant difference of the hatching time was observed between these two groups. The hatchabilities of groups in which the respiratory area was blocked (groups 2, 3 and 5) were much less than the control and the hatchability was not improved by opening the air space to the atmosphere (group 5). From these facts as well as the blood gas properties measured, we can also assess the following results. 1) The blood gas tensions both in groups 2 and 3 were almost the same throughout the experimental period and no significant difference in hatchability was observed between these two groups. The facts imply that the gas exchange through the shell both over the narrow end and the broad end is performed only in proportion to the total respiratory shell area. Therefore, a respiratory advantage of the air space could not be considered to exsist. 2) The death toll in groups whose blood P_{O_2} was greatly diminished was large. This tendency was observed even in group 5 in which blood P_{CO_2} was diminished by increasing the diffusing rate at the site of the air space. In this group, the concentration of gases in the air space was equal to that of the atmosphere. Although the blood O_2 content in embryo increases during development, diminished blood P_{O_2} may not be sufficient to transport the required O_2 in these groups in which the respiratory area on the shell was reduced. Therefore, the possible cause of the death in the shell before pipping might be the O_2 deficit in blood and accordingly in the tissue. 3) CO_2 accumulated in the air space and blood might not play a special role for pipping the shell, because the eggs in group 4 normally pipped and hatched despite extremely low P_{CO_2} in the air space and blood. 4) It appears that a pipping stimulus can not always be attributed to the gases in the air space and blood, although it may be true in some cases where the high level of P_{CO_2} and low P_{O_2} accelerate the pipping (VISSCHEDIJK, 1968).

TEMPERATURE EFFECTS ON THE BLOOD GAS EXCHANGE

The embryonic development is wholly dependent on the temperature of incubation. The rate of development increases with the rising temperature until an optimum is reached. Fig. 3 shows the changes in the embryonic weights during development. The eggs were incubated either at temperature of 38°C or 35°C. Development was manifestly retarded at 35°C incubative temperature and the embryos required 25 to 26 days for hatching. Naturally,

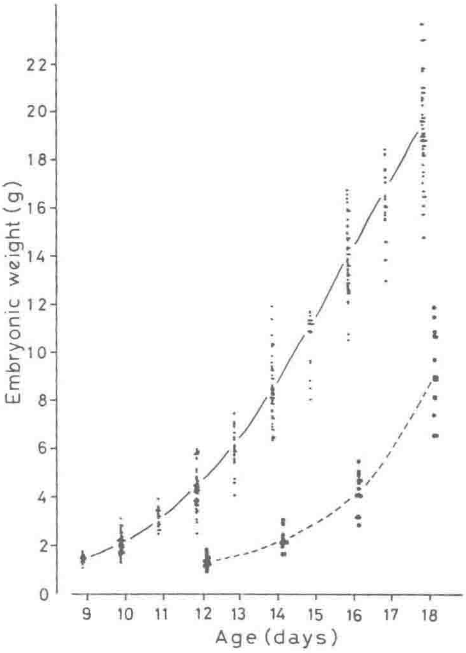

Fig. 3. Changes in the embryonic weight plotted against the days of incubation. The small dot indicates the weight of embryo incubated at 38°C and the large dot, the weight of embryo incubated at 35°C. The eggs incubated at 35°C required approxiately 25 days for hatching and the hatchability became less.