REVISTA ESPAÑOLA DE FISIOLOGIA, 40, 411-416. 1984

Effects of Acute Hypobaric Hypoxia on Acid-Base Status of Fowl Blood

A. F. Sigüenza, M. D. Córdova, M. T. Agapito and J. M. Recio

Cátedra de Fisiología Animal
Facultad de Ciencias
Universidad de Valladolid
Valladolid (Spain)

(Received on September 2, 1983)

A. F. SIGÜENZA, M. D. CORDOVA, M. T. AGAPITO and J. M. RECIO. *Effects of Acute Hypobaric Hypoxia on Acid-Base Status of Fowl Blood.* Rev. esp. Fisiol., **40**, 411-416, 1984.

Arterial blood Po_2, Pco_2, lactate levels and Cl^- ion concentration as well as pH were measured on the time course in chickens (*Gallus domesticus*) as they settled in normoxic conditions and during exposure to acute hypobaric hypoxia (P_b = 450 Torr).

Hypoxia provoked at first a CO_2 increased output from blood and a brief stage of deep metabolic acidosis during which lactate levels suddenly increased. This acidosis was then compensated producing a return to the initial pH and a decrease in $[HCO_3^-] + [CO_3^{=}]$ after 60 min. Subsequently respiratory alkalosis associated with an increase in $[HCO_3^-] + [CO_3^{=}]$, a decrease in Cl^- ion concentration and a small decrease in lactate levels were observed. Prolonged exposure to hypoxia (16 h) resulted in a new return to the initial pH, a decrease in concentration of $[HCO_3^-] + [CO_3^{=}]$ and a high lactate level.

The hematocrit value, the Hb concentration, and the plasma Na^+, K^+, Ca^{++} and Mg^{++} ion concentration did not change significantly.

Key words: Hypoxia, Acid-Base, Fowl.

Little is known about the blood gases of domestic fowl, and the knowledge of acid-base status is less complete than that of many other domestic animals (8). Few reports have appeared in the past concerning the normal arterial saturation, arterial Po_2 and Pco_2 and oxygen dissociation curve (6, 13, 15). HELBACKA and CASTERLINE (10) have determined if a relationship exists between acid-base status and egg quality factors. Arterial chemoreceptors in ventilatory adjustments to hypoxia in ducks have been described (2, 3).

However, responses to hypoxia exposure are not known in domestic hen. Thus the present study examines the effects of hypoxia on arterial blood gases, acid-base parameters and Cl^-, Na^+, K^+, Ca^{++} and Mg^{++} ion concentration under normoxic and hypoxic conditions.

Materials and Methods

Experiments were performed on 15 male of the White Rock breed weighing 1.7-2.6 kg. The birds were housed and kept in cages under natural conditions and photoperiod, with standard dry food and water *ad libitum*. Previous to experiments, a plastic catheter (1 × 70 mm) was implanted in the braquial artery. The cannulation was carried out on animal anesthetized by intramuscular injection of sodium pentobarbital (50 mg/kg). A minimum of one day was allowed for the operated birds to recover completely from surgery before experiments started, since the injection of sodium pentobarbital diminished the tolerance of the animals to acute hypoxia (13). When the cannula had been secured, 2 ml heparin solution, 10 mg/ml in saline was injected into the artery. Pan-Terramicin Pfizer was used to avoid infeccions.

The awake animal sat in a wood box with the head protruding through an opening in order to prevent the bird from removing the cannula. 100 μl of blood was collected in a heparinized capillary, immediately capped and stored in ice. The experimental bird was placed in a low pressure chamber constructed for us, and exposed to a simulated altitude of 4,300 m (P_b = 450 Torr). This altitude was maintained 1/2, 1, 3 and 16 h. Blood gas tensions and pH were measured in a IL 213 microelectrode thermostated analyzer at 42° C. Bicarbonate plus carbonate concentration ($[HCO_3^-] + [CO_3^{2-}]$) in plasma was calculated with the aid of the Henderson-Hasselbach relationship, assuming pK' = 6,10, and vol % dissolved CO_2 = 0.0289 mmol.l^{-1}. Torr^{-1} at 42° C. Lactate in plasma and chloride were measured by the enzymatic method of HOHORST (12) and Slyke and Sendroy method described by VILALLONGO *et al.* (22) respectively. Microhematocrit value and hemoglobin concentration were determined on a small blood sample drawn from the brachial artery. Hemoglobin concentrations were determined by the cyanmethemoglobin method (7).

Cations Na^+, K^+, Ca^{++} and Mg^{++} were measured by Atomic Absorption Spectrophotometric (Perkin Elmer mod. 300).

The significance $P < 0.05$ of the changes occurring after hypoxia was tested using Student's t test.

Results

Table I and figure 1 summarize the results obtained during hypoxia. During the first 30 min of hypoxia, pH and Pco_2 fall and concomitantly the ventilatory frequency increases. Simultaneously there is a marked drop in $[HCO_3^-] + [CO_3^{2-}]$ and a significant increase in lactate level.

At the first hour of hypoxia an increase in pH can be observed, which reflects a modification of the acid-base balance in the direction of a respiratory alcalosis, while Pco_2 and $([HCO_3^-] + [CO_3^{2-}]$ decrease with respect to initial values. During this period it is possible to observe a significant increase in lactate level.

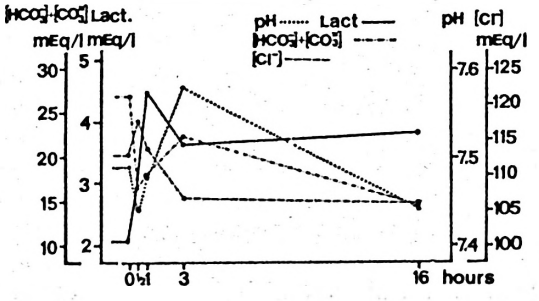

Fig. 1. *$[HCO_3^-] + [CO_3^{2-}]$ pH, lactate and Cl^- changes as a function of time during a stage in hypoxia (P_b = 450 Torr) in* Gallus domesticus. *Temperature 42° C.*

Table I. *Arterial blood characteristics of* Gallus domesticus *at 42° C and changes produced during 16 h of acute hypobaric hypoxia (P_b = 450 Torr).* Values are means ± SE. PaO_2 and $PaCO_2$, arterial O_2 and CO_2 partial pressures; SaO_2, O_2 arterial saturation; pH_a, arterial pH. * Not significantly different from initial value, $P < 0.05$, n = number of animals. Mean values of the other parameters: Hemoglobin = 10.0 ± 0.9 g/100 ml, Hematocrit = 30.9 ± 1.5 %, Na^+ = 142.4 ± 12.6 mEq/l, K^+ = 4.21 ± 0.67 mEq/l, Ca^{++} = 9.42 ± 1.11 mEq/l and Mg^{++} = 1.23 ± 0.26 mEq/l.

	n	Pa_{O_2} Torr	SaO_2 %	Pa_{CO_2} Torr	pH_a	$HCO_3^- + CO_3^{=}$ mEq/l	Lactate mEq/l	Cl^- mEq/l
Normoxia t = 0 h	13	85.2 ± 3.2	86.3 ± 2.8	34.9 ± 2.3	7.49 ± 0.020	27.0 ± 1.3	2.07 ± 0.28	113.0 ± 2.8
Hypoxia t = ½ h	7	71.9 ± 1.8	76.2 ± 1.9	24.9 ± 1.2	7.44 ± 0.005	16.5 ± 0.7	3.06 ± 0.14	118.7 ± 6.1 *
Hypoxia t = 1 h	6	76.4 ± 1.6	81.3 ± 1.4	24.1 ± 1.0	7.48 ± 0.034 *	17.9 ± 1.3	4.48 ± 0.43	114.5 ± 2.8 *
Hypoxia t = 3 h	5	75.4 ± 1.2	80.0 ± 1.6	24.7 ± 1.2	7.58 ± 0.022	22.5 ± 1.8	3.64 ± 0.36	107.0 ± 3.2
Hypoxia t = 16 h	5	72.4 ± 1.2	77.1 ± 1.3	22.4 ± 2.3	7.44 ± 0.038	14.6 ± 0.8	3.81 ± 0.47	106.0 ± 2.7

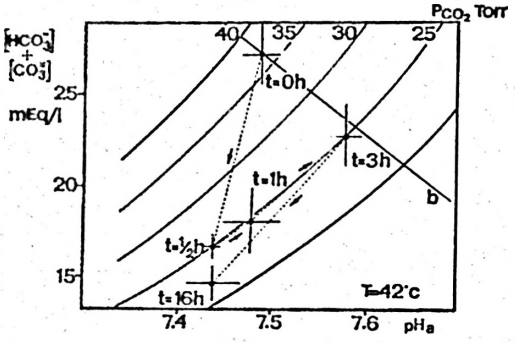

Fig. 2. *Diagram of* $[HCO_3^-] + [CO_3^{2-}]$ *vs pH changes in arterial blood of* Gallus domesticus *during 16 h hypoxia.*
Calculated Pco_2 isopleths are drawn on the diagram. Hypoxia times in hours. Vertical and horizontal bars represent standard error. The oblique line represents mean buffer line (b) calculated from two in vitro pH measurements at different Pco_2. The arrows indicate the time course of changes observed in acid-base status. Temperature 42° C.

After three hours of hypoxia both lactate and Cl^- ion concentration fell and an associated rise in $[HCO_3^-] + [CO_3^{2-}]$ ocurred at this time. At the end of 16 h of hypoxia the lactate concentration remains higher than its reference value and $[HCO_3^-] + [CO_3^{2-}]$ remain lower than the level in normoxia.

The effect of such treatment on the acid-base status of the blood is indicated in figure 2 in which the concentration of bicarbonate plus carbonate is plotted against pH.

Neither hemoglobin concentration and hematocrit nor Na^+, K^+, Ca^{++} and Mg^{++} ion concentration changed significantly under hypoxia.

Discussion

Normoxic studies. The average arterial blood pH values in domestic fowls were more alkaline than in most homeotherms. These results agree with those of CHIODI and TERMAN (5) in awake domestic hen. The Pco_2 values are similar to those observed by MORGAN and CHICHESTER (13) but higher than those found in a previous report (5). The $[HCO_3^-] + [CO_3^{2-}]$ from Leghorn hen plasma was estimated between 21-28 mEq/l (1,16).

Then mean lactate concentration was higher than the one given by others in domestic hen (5) and human plasma (18).

The arterial saturation and Po_2 were slightly lower than the values reported by MORGAN and CHICHESTER (13). Hematocrit value agree with those calculated by CHIODI and TERMAN (5) and STURKIE (17).

The Na^+, K^+, Ca^{++}, Mg^{++} and Cl^- concentration were slightly higher than those found in the literature (9, 11, 17).

Hypoxia. Literature data about the effects of hypoxia on acid-base status are somewhat confusing since hypocapnic alkalosis induced by hyperventilation and lactacidemia are frequently intermingled. Results obtained in this investigation show clearly that the effects observed depend on the time at which measurements are performed. At the onset of hypoxia, birds show a response characterized by an initial rise in ventilatory frequency associated with an increase in ventilatory volume. The increased ventilation leads to a greater difference in CO_2 tension between the blood and the air across the lung, that enhances the release of this gas. This initial response seems to be reflexly induced by the arterial chemoreceptors (2).

During the first 30 min, there is a decrease in blood pH concomitant with a drop in plasma $[HCO_3^-] + [CO_3^{2-}]$. This pH compensatory mechanism occurs at constant blood Pco_2 and consequently has no ventilatory origin. It may involve either a change in plasma ionic composi-

tion or an appearance of metabolic acids in the blood. Excess lactate is a useful quantifier of tissue hypoxia (4). The reactions of a noncarbonic acid, as lactic acid, with bicarbonate buffer produces CO_2 that can be dissipated at the respiratory organs. On interaction with the blood proteins, the product HX accumulates in the blood restaining further reaction of X with H.

The drop in bicarbonate level on the Pco_2, pH diagram (fig. 2), makes it possible to predict the amplitude of the decrease attributable to the release of lactic acid. To estimate quantitatively the effect on blood pH of the increase from 2.07 to 4.48 mEq/l in lactate level, a computing model suggested by STEWART (18) has been used (table I). ZBOROWSKA-SLUIS and DOSSETOR (23) showed that pH rise stimulated glycolysis in red blood cells with an increase in lactate production, and the effect of pH on arterial lactate increase is greater than on excess lactate in the presence of an oxygen deficit.

Results show some significant modifications in plasma Cl^- ion concentration, suggesting the intervention of a $Cl^- \rightleftharpoons HCO_3^-$ exchange process. The drop in $[HCO_3^-] + [CO_3^{2-}]$ can be partially compensated by a change in plasma Cl^- ion concentration, in the same way as observed by THOMAS *et al.* (20) in hypocapnic conditions which lead to similar changes in acid-base balance.

Resumen

Se determina en sangre arterial de pollos (*Gallus domesticus*) las Po_2 y Pco_2, el pH, niveles de lactato y concentración de Cl^- en condiciones de normoxia e hipoxia hipobárica aguda (P_b = 450 Torr). La hipoxia provoca inicialmente un incremento en la pérdida del CO_2 sanguíneo y un breve estado de acidosis metabólica profunda durante el cual los niveles de lactato aumentan de forma súbita. Esta acidosis se compensa luego con restablecimiento del pH inicial y descenso en la $[HCO_3^-] + [CO_3^{2-}]$ a los 60 minutos. Posteriormente aparece una alcalosis respiratoria asociada a un aumento en la $[HCO_3^-] + [CO_3^{2-}]$, descenso en la concentración del Cl^- y ligero descenso en los niveles de lactato. A las 16 horas de hipoxia se observa restablecimiento del pH, descenso en la $[HCO_3^-] + [CO_3^{2-}]$ y nivel aún alto de lactato. El valor del hematocrito, la concentración de Hb y la concentración en plasma de Na^+, K^+, Ca^{++} y Mg^{++} no presentan cambios significativos.

References

1. ACKENSON, BLISH and MUSSEHL: *J. Biol. Chem.*, **63**, 75, 1925.
2. BOUVEROT, P., DOUGUET, D. and SEBERT, P.: *J. Comp. Physiol.*, **133**, 177-186, 1979.
3. BOUVEROT, P. and HILDWEIN, G.: *Res. Physiol.*, **35**, 373-384, 1978.
4. CAIN, S. M.: *J. Appl. Physiol.*, **42**, 44-49, 1977.
5. CHIODI, H. and TERMAN, J. W.: *Am. J. Physiol.*, **208**, 798-800, 1965.
6. CHISTENSEN, E. H. and DILL, D. B.: *J. Biol. Chem.*, **109**, 443-448, 1935.
7. CROSBY, W. H., MUNN, J. J. and FURTH, F. W.: *U. S. Armed Forces Med. J.*, **5**, 693-703, 1954.
8. DUKES, H. H.: In «The Physiology of Domestic Animals» (6th ed.). Comstock Publ. Ass., Ithaca, 1947.
9. DYER, H. M. and ROE, J. H.: *J. Nutr.*, **7**, 623, 1943.
10. HELBACKA, N. V. and CASTERLINE, J. L.: *Poultry Sci.*, **42**, 450-451, 1963.
11. HELLER, V. G. and PURSELL, L.: *J. Biol. Chem.*, **118**, 549, 1973.
12. HOHORST, J. M.: In «Methods of Enzymatic Analysis» (H. U. Bergmeyer, ed.). Academic Press, New York, 1981, p. 266.
13. MORGAN, W. E. and CHISCHESTER, D. F.: *J. Biol. Chem.*, **110**, 285-298, 1935.
14. QUATRINI, U. and BENIGNO, A.: *Boll. Soc. ital. Biol. Sper.*, **57**, 333-339, 1981.
15. ROETERFER, H. H. and RIGDON, R. H.: *Biol. Bull.*, **92**, 23-30, 1947.
16. SHIMER, S. R.: *Tech. Bull.*, **1**, 69, 1937.
17. STURKIE, P. D.: Avian Physiology (2nd ed.). Comstock Publ. Ass., Ithaca, 1965.
18. STEWART, P. A.: *Resp. Physiol.*, **33**, 9-26, 1978.

19. SYKES, A. H.: *Poultry Sci.*, **39**, 16-17, 1965.
20. THOMAS, S., BELAN, A., BARTHELEMY, L. and PEYRAND, C.: *J. Comp. Physiol.*, **140**, 249-254, 1980.
21. THOMAS, S. and HUGHES, G. M.: *Resp. Physiol.*, **49**, 371-382, 1982.
22. VILALLONGO, F. A., MARENZI, A. D., CARCINI, C. E. and BONFI, R. F.: Bioquímica Analítica Cuantitativa. Ateneo, Córdoba (Argentina), 1947.
23. ZBOROWSKA-SLUIS, D. T. and DOSSETOR, J. B.: *J. Appl. Physiol.*, **22**, 746-755, 1967.