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Body Fluid Compartment Volumes in Chronically Hypoxemic Lambs[1]

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ABSTRACT. Chronic hypoxemia is associated with a decreased growth rate and a decreased rate of cell division. In lambs with experimental cyanotic heart disease, the gain in body mass was decreased. In this study, we determined to what extent the lower body mass in these lambs was related to alterations in body fluid compartment volumes, specifically whether intracellular volume was decreased. Therefore, fluid compartment volumes were studied in nine lambs, after 3 to 4 wk of experimental cyanotic heart disease, and in 13 control lambs. Hypoxemic lambs had a lower arterial oxygen saturation (65 ± 11 *versus* 91 ± 2%, $p < 0.001$) and a higher Hb concentration (142 ± 16 *versus* 101 ± 8 $g \cdot L^{-1}$, $p < 0.001$). The lower body mass in hypoxemic lambs (10.5 ± 2.3 *versus* 13.0 ± 2.8 kg) could be mainly accounted for by a decrease in intracellular water volume (4.7 ± 1.3 *versus* 6.6 ± 1.5 L, $p < 0.01$). Total body water (753 ± 27 *versus* 780 ± 40 $mL \cdot kg^{-1}$) and extracellular water volume (307 ± 25 *versus* 277 ± 15 $mL \cdot kg^{-1}$) in hypoxemic lambs were not significantly different from those in control lambs, but intracellular water volume was decreased (445 ± 27 *versus* 501 ± 35 $mL \cdot kg^{-1}$, $p < 0.01$). The ratio of extracellular to intracellular water volume was higher in hypoxemic lambs (0.69 ± 0.10 *versus* 0.55 ± 0.04, $p < 0.01$). Blood volume was increased in hypoxemic lambs (121 ± 29 *versus* 79 ± 15 $mL \cdot kg^{-1}$, $p < 0.01$), mainly due to an increased total red cell volume (48 ± 12 *versus* 22 ± 8 $mL \cdot kg^{-1}$, $p < 0.001$). We conclude that the lower body mass in hypoxemic lambs is mainly related to a lower intracellular water volume. These results and the increased ratio of extracellular to intracellular water volume can be explained by assuming a decreased growth of the intracellular compartment. In addition, a part of the (decreased) growth in hypoxemic lambs must be expended on increased erythropoiesis. (*Pediatr Res* 33: 267–272, 1993)

Abbreviations

V_{ec}, extracellular water volume
V_{ic}, intracellular water volume
D_2O, deuteriumoxide
i.m., intramuscularly

Congenital heart disease is often associated with a decreased growth rate, characterized by a decreased gain in body mass and length (1–3). It seems to affect children with cyanotic heart disease more than children with noncyanotic heart disease (1–3). In young experimental animals exposed to various forms of chronic hypoxemia, a decreased body mass is a common finding (4–8). Based on studies in rats and mice, it has been suggested that a decreased rate of cell division is responsible for the growth retardation (4, 5).

The volumes of body fluid compartments change during growth. Both V_{ec} and V_{ic} increase, but per-unit body mass total water and V_{ec} decrease and V_{ic} increases (9, 10). This can in part be explained by a relatively faster growth of the intracellular compartment (9, 11). In chronic hypoxemia, therefore, body fluid compartment volumes may be altered by the effects of decreased growth.

Another factor that may affect fluid compartment volumes is the effect of chronic hypoxemia on blood volume. Blood volume increases through an increase in red cell volume, whereas plasma volume is either normal or decreased (12, 13).

Fluid compartment volumes and blood volume have been measured in chronic hypoxemia. However, to our knowledge, the effect of cyanotic heart disease on fluid compartment volumes and on blood volume has not been measured simultaneously in young, growing subjects. Therefore, we measured total body water volume, V_{ec}, and plasma volume in lambs that had been hypoxemic for 3 to 4 wk due to experimental cyanotic heart disease, and we calculated V_{ic}, interstitial volume, and blood volume. Thus, the relation between the lower body mass found in these lambs (7) and alterations in fluid compartment volumes, especially the V_{ic}, was studied, and the alterations in blood volume in relation to other fluid compartment volumes was determined.

MATERIALS AND METHODS

We studied nine chronically hypoxemic and 13 control lambs, 5 to 6 wk old, of mixed breed. All hypoxemic lambs underwent surgery before the 10th day of life. Control lambs either underwent surgery at least 2 wk (14–34 d) before the measurements ($n = 4$), 1 wk (5–8 d) before the measurements ($n = 4$), or had neck vessel catheters inserted 3 to 7 d before the measurements ($n = 5$).

Surgical procedure and postoperative care. Anesthesia was induced by 2 to 3% halothane in oxygen. The lamb was placed on a warming pad (39°C), intubated, and ventilated with a mixture of halothane (0.5–1.5%), oxygen (40–60%), and room air by a Servo Ventilator 900B (Siemens-Elema AB, Solna, Sweden). Analgesia was maintained with piritramide 10 to 20 mg i.m., and lidocaine hydrochloride (5 $g \cdot L^{-1}$) was administered locally before each skin incision.

The left thoracic cavity was opened in the 4th intercostal space.

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Polyvinyl catheters (outside diameter 1.5 mm, inside diameter 1.0 mm) were inserted into the ascending aorta through the internal thoracic artery and into the superior vena cava through the internal thoracic vein. The pericardial sac was opened, and catheters were inserted through purse-string sutures into the pulmonary artery, the outflow tract of the right ventricle, and the left atrium. In the lambs that were to be made hypoxemic, an atrial septostomy was performed by means of a 5 F balloon-tipped Fogarty catheter (American Edwards Laboratories, Santa Ana, CA) introduced through a pedal vein. After positioning the tip into the left atrium through the foramen ovale, we inflated the balloon with 1.5 to 2.0 mL sterile saline solution and rapidly withdrew it into the right atrium, thus tearing the atrial septum. This procedure was repeated two to three times. An inflatable silicone rubber constrictor, inside diameter 8 to 10 mm (Hazen Everett Co., Teaneck, NJ), was fitted around the main pulmonary artery. An 8 F polyvinyl catheter was placed to drain the left thoracic cavity. All catheters were tunneled to the left flank 5 to 10 cm caudal of the 4th intercostal space. The thorax was closed in layers and all the catheters, except the one for chest drainage, were filled with a heparin solution (1000 U/mL). All catheters were sealed and protected in a Teflon pouch that was attached to the skin. Eight lambs that served as controls underwent the same surgical procedure apart from the atrial septostomy, the constrictor around the pulmonary artery, and the insertion of a right ventricular catheter. The left thoracic cavity was aspirated daily for 4 to 6 d, then the thoracic drain was removed. Daily, the lambs were weighed and catheters refilled with fresh heparin solution.

Three to 5 d after surgery, the constrictor around the pulmonary artery was inflated with sterile saline solution (9 $g \cdot L^{-1}$), thus inducing an atrial right to left shunt through the foramen ovale. On the 1st and 2nd day of inflation, the right ventricular systolic pressure was raised to systemic and suprasystemic levels, respectively. Thereafter, the constrictor was inflated to lower the arterial oxygen saturation to 60 to 70% and to keep it within this range.

Neck vessels were catheterized by incising the skin over the right carotid artery and jugular vein under local anesthesia. A balloon-tipped catheter that was connected to a pressure transducer was inserted into the jugular vein and positioned into the pulmonary artery while a pressure tracing was monitored. The right carotid artery was ligated, and a catheter was advanced to the ascending aorta. All lambs were given 150 mg ampicillin i.m. for 4 d after surgical procedures. Weekly, each lamb was given iron dextran complex i.m. equivalent to 200 mg iron.

Experimental protocol. Plasma volume, V_{ec}, and total body water volumes were determined by means of a single-injection triple-indicator dilution technique. A freshly prepared mixture of indicators was injected into the pulmonary artery. Just before the injection (time zero), and at 3, 6, 9, 12, 15, 20, 25, 30, 40, 50, 60, 75, 90, 105, 120, 135, 150, 165, and 180 min after the injection, 2- to 4-mL blood samples were withdrawn from the aorta into dry-heparinized tubes. Hematocrit of the blood samples taken at 0, 20, 30, 40, 50, and 60 min was determined in duplicate. Before time zero, blood was obtained to measure plasma osmolality and total protein concentration. After the withdrawal of each blood sample, a similar volume of sterile saline (9 $g \cdot L^{-1}$) was injected into the pulmonary artery; a total volume of 60 to 70 mL blood was withdrawn during the experiment.

Measurements and calculations. One $mL \cdot kg^{-1}$ of a mixture of 0.4 $mmol \cdot L^{-1}$ Evans Blue (Merck, Darmstadt, Germany), 100 $mmol \cdot L^{-1}$ sodium ferrocyanide (BDH Chemicals, Poole, England) in D_2O 99.8% (Merck) was injected to determine the plasma volume, the V_{ec}, and the total body water volume, respectively. The exact amount of indicator injected was determined by weighing the syringe containing the indicator mixture before and after the injection. After treatment of the samples as described below, light absorbances were measured and converted to concentrations by using a calibration line that was determined before each set of measurements as described by Zweens *et al.* (14–16).

The Evans Blue concentration was measured in the samples withdrawn at 20, 30, 40, 50, and 60 min. Polyethylene glycol (240 $g \cdot L^{-1}$; 0.8 mL) was mixed with plasma (0.8 mL) and allowed to stand for 10 min. The mixture was then centrifuged at 7000 × *g* for 10 min, and the absorbance of the supernatant was measured at 620 nm with a Hitachi 100-40 spectrophotometer (Hitachi Ltd., Tokyo, Japan) against a similarly treated plasma blank. The concentration at time zero (c_0^{EB}) was calculated by extrapolation from the monoexponential concentration *versus* time curve. Because Evans Blue is completely mixed with plasma 10 min after injection and subsequently disappears from plasma in a first-order fashion, the error made in c_o^{EB} by assuming monoexponential elimination between 20 and 60 min after injection is negligible (16). The plasma volume (V_p) was calculated as:

$$V_p = m_i^{EB}/c_0^{EB} \quad (1)$$

where m_i^{EB} denotes the amount of Evans Blue injected.

The sodium ferrocyanide concentration was determined in all samples. A solution (4.5 mL) containing trichloric acetic acid (0.14 mmol/L) and perchloric acid (1.10 mmol/L) was mixed with 0.5 mL plasma and allowed to stand for 10 min. Subsequently, it was centrifuged for 10 min at 7000 × *g*; then 1 mL of a solution containing $FeSO_4$ (5 g/L) and H_2SO_4 (90 mmol/L) was added to 4 mL of supernatant. After 25 min, a blue color had developed that remained stable for at least 15 min. During this period, the absorbance of the solution was measured against a reagent blank at 700 nm by means of an Optica CF4 spectrophotometer (Optica, Milan, Italy). The plasma concentrations (c_p^{FC}) were converted to plasma-water concentrations (c_{pw}^{FC}), by correcting for plasma protein concentration

$$c_{pw}^{FC} = c_p^{FC} \cdot 1000/(1000 - 0.75 \cdot c_p^{TP}) \quad (2)$$

where c_p^{TP} is total plasma protein concentration (g/L) and 0.75 is the specific volume of protein (mL/g). The time-concentration curve obeyed a triexponential model:

$$c_t = Ae^{-k_1 t} + Be^{-k_2 t} + Ce^{-k_3 t} \quad (3)$$

where A, B, and C are the coefficients, and k_1, k_2, and k_3 the exponents of each of the monoexponential parts of the curve, which can be determined by curve stripping. The V_{ec} was then calculated:

$$V_{ec} = m_i^{FC} \frac{(A/k_1^2 + B/k_2^2 + C/k_3^2)}{(A/k_1 + B/k_2 + C/k_3)^2} \quad (4)$$

The symbols are identical to those in equation 3, and m_i^{FC} denotes the amount of sodium ferrocyanide injected.

To determine the D_2O concentration, about 0.5 mL of red cells were vacuum-sublimated to near dryness, and the condensate was trapped in tubes immersed in liquid nitrogen. The absorbance of the condensate was determined against a reference of ordinary window glass at 4023 nm by a Perkin-Elmer 177 infrared-spectrophotometer (Perkin Elmer Corp., Norwalk, CT) (15). Because the distribution phase of D_2O is short (≈40 min) relative to the elimination phase ($t_{½} \approx 6$ d) (15), $c_0^{D_2O}$ can be calculated assuming monoexponential elimination. In four lambs, D_2O concentrations were determined in all samples, and no observable change in D_2O concentration was found after 40 min. Therefore, in the subsequent experiments, we calculated $c_0^{D_2O}$ as the mean of the concentrations at 50 to 120 min. Body water volume (V_{bw}) was calculated as:

$$V_{bw} = m_i^{D_2O}/c_0^{D_2O}$$

where $m_i^{D_2O}$ denotes the amount of D_2O injected.

Plasma osmolality was determined in duplicate by means of an Osmomat 030 (Gonotec GmBH, Berlin, Germany), concen-

tration of total protein by an automatic chemical analyzer ACA III (DuPont Co., Wilmington, DE) and concentration of sodium by flame photometry (IL343, Instrumentation Laboratory Inc., Lexington, MA). Hematocrit was determined by the microhematocrit method; the centrifugation time was 7 min. Because ferrocyanide is freely filtered by the glomeruli and neither excreted nor reabsorbed by the tubules, the GFR was calculated from the slope of the monoexponential tail of the disappearance curve (14).

Blood volume (V_b) was calculated as $V_p \times 100/(100 - \text{hematocrit} \times 0.92)$; the factor 0.92 corrects for the difference between the arterial and total body hematocrit (12). The total red cell volume ($V_{\text{red cell}}$) was calculated as $V_b - V_p$; the total amount of Hb as V_b (L) × Hb concentration ($g \cdot L^{-1}$); the interstitial water volume (V_{int}) as $V_{ec} - V_p$; the V_{ic} as $V_{bw} - V_{ec}$; and, the mass of the solids, as body mass − total body water mass, assuming a density of water of 1.00 $kg \cdot L^{-1}$. To determine the effects of hypoxemia on V_{ic}, not related to alterations in red cell mass, we calculated the intracellular volume that was extravascular ($V_{ic}^{\text{extravascular}}$):

$$V_{ic}^{\text{extravascular}} = V_{ic} - (V_{\text{red cell}} - V_{\text{hemoglobin}})$$

$V_{\text{hemoglobin}}$ was calculated assuming a similar specific volume of Hb and of plasma protein (0.75 mL/g).

Statistical analysis. The mean and SD of each variable were calculated. Control lambs were divided into three groups: a group that underwent surgery more than 2 wk before the experiment ($n = 4$), a group that had surgery 5 to 8 d before the experiment ($n = 4$), and a group that underwent neck vessel catheterization ($n = 5$). The data of the three groups of control lambs and those of hypoxemic lambs were analyzed by means of one-way analysis of variance with a statistical program (NCSS, J.L. Hintze, Kaysville, UT). When the F value was higher than the critical value, multiple contrasts were used to test for the effects of surgery, timing of surgery, and hypoxemia. Therefore, the following subgroups were compared: the two groups of control lambs that underwent surgery ($n = 4$ in each group), control lambs that underwent surgery ($n = 8$) *versus* those that underwent neck vessel catheterization ($n = 5$), and control ($n = 13$) *versus* hypoxemic lambs ($n = 9$). If no differences between the subgroups of control lambs were found, an unpaired t test was performed between control and hypoxemic lambs. Unless otherwise stated, the results of analysis of variance are reported. Linear regression was used to analyze body fluid volume ratios as a function of body mass. In all analyses, $p < 0.05$ was chosen as the level of significance.

RESULTS

Age, body mass, and fluid compartment volumes per unit mass in the three groups of control lambs and in hypoxemic lambs are shown in Table 1. Because no differences were found in any of these variables between the three groups of control lambs, the data of all control lambs were pooled.

Hypoxemic lambs were studied 26 ± 3 d after the induction of hypoxemia. Their arterial oxygen saturation was decreased (65 ± 11 *versus* 91 ± 2%, $p < 0.001$), and their Hb concentration was increased (142 ± 16 *versus* 101 ± 8 $g \cdot L^{-1}$, $p < 0.001$).

Body mass of hypoxemic and control lambs and the division in body compartments are shown in Figure 1. Body mass and V_{ic} were lower in hypoxemic lambs, whereas the V_{ec} and the mass of solids were similar in hypoxemic and control lambs. The difference in body mass between hypoxemic and control lambs could almost completely be accounted for by the difference in total body water volume (7.9 ± 1.8 *versus* 10.3 ± 2.3 L, $p < 0.02$, t test).

Per-unit body mass total body water was similar in hypoxemic and control lambs, but V_{ec} was higher in hypoxemic lambs, and V_{ic} was lower (Table 1). V_{ec}/V_{ic}, the ratio of V_{ec} to V_{ic} (Fig. 2), decreased with body mass in hypoxemic lambs ($y = -0.03x + 1.04$, $r = 0.81$, $p < 0.01$) but did not change with body mass in control lambs ($y = 0.003x + 0.52$, $r = 0.10$). V_{ec}/V_{ic} was higher in hypoxemic than in control lambs (0.69 ± 0.10 *versus* 0.55 ± 0.04, $p < 0.01$). Plasma osmolality (281 ± 10 *versus* 286 ± 4 $mmol \cdot L^{-1}$), total protein concentration (61 ± 7 *versus* 59 ± 5 $g \cdot L^{-1}$), sodium concentration (144 ± 4 *versus* 145 ± 2 $mmol \cdot L^{-1}$), and GFR (2.7 ± 0.5 *versus* 2.7 ± 0.4 $mL \cdot min^{-1} \cdot kg^{-1}$) were similar in hypoxemic and control lambs. Blood volume (Fig. 3) was increased in hypoxemic lambs because of an increased total red cell volume. The total amount of Hb in hypoxemic lambs was increased 2-fold (177 ± 51 *versus* 97 ± 28 g, $p < 0.01$).

The V_{ic} that was extravascular was lower in hypoxemic lambs (415 ± 29 *versus* 489 ± 34 $mL \cdot kg^{-1}$, $p < 0.001$). The ratio of interstitial water volume to the extravascular part of V_{ic}, which is the extravascular equivalent of V_{ec}/V_{ic}, was also increased in hypoxemic lambs (0.57 ± 0.07 *versus* 0.45 ± 0.05, $p < 0.001$).

DISCUSSION

The present study demonstrates that 3 to 4 wk of chronic hypoxemia in lambs, due to experimentally induced cyanotic heart disease, has two distinct effects on body fluid compartment volumes. First, blood volume is increased mainly through an increased red cell mass. Second, V_{ic} is decreased, which almost completely accounts for the difference in total body water volume and body mass between hypoxemic and control lambs. Because total water volume per unit of mass is similar in hypoxemic and control lambs, a larger fraction of body water is distributed to the extracellular space in hypoxemic lambs, which is demonstrated by the increased ratio of V_{ec} to V_{ic}.

We found no differences between the three subgroups of control lambs (Table 1), indicating that surgery or the timing of surgery did not affect body fluid compartment volumes. Although the number of lambs in each subgroup is small, the data of the control lambs are very similar to each other and in contrast with those of hypoxemic lambs. When a t test is applied, more differences between control and hypoxemic lambs become apparent. For example, body mass is lower in hypoxemic lambs, which is in accordance with the decreased gain in body mass that has been shown in hypoxemic lambs by Teitel *et al.* (7).

Alterations in body fluid compartment volumes in (congenital) heart disease may be related to water and salt retention (17, 18). However, the alterations in fluid compartment volumes in the hypoxemic lambs in our study are not readily explained by this mechanism. Water and salt retention is associated with an increase in total body water and V_{ec}, whereas V_{ic} is not decreased (19). In dogs with an experimental pulmonary stenosis, no signs of venous congestion or water and salt retention were found (20, 21). The hypoxemic lambs in our study had no signs of venous congestion either. Water and salt retention in chronic obstructive lung disease was related to hypercapnia rather than to hypoxemia (22, 23). In the early phase of (simulated) high-altitude hypoxemia, voluntary sodium and water intake are decreased, and natriuresis and diuresis are increased (24). In chronically hypoxemic rats, GFR and urinary sodium excretion were normal (25), similar to the GFR in our study. It is improbable that body water in hypoxemic lambs was redistributed due to osmotic shifts, because plasma osmolality, sodium, and total protein concentrations were not increased. Thus, water and salt retention or an osmotic shift of water from the intracellular to the extracellular compartment cannot explain the differences in fluid compartment volumes between hypoxemic and control lambs.

Fluid compartment volumes per unit body mass change during growth. After birth, total body water and V_{ec} decrease and V_{ic} increases (9, 10). These changes have been attributed both to a relative loss of body water and to growth of the intracellular compartment. In infants, a redistribution of water from the extracellular to the intracellular compartment was found in the first 2 wk of life, and this was attributed to the effects of cellular

Table 1. *Fluid compartment volumes in control and hypoxemic lambs**

	Control/surgery (>2 wk)	Control/surgery (<1 wk)	Control/no surgery	Control/pooled data	Hypoxemia	*t* test (*p* value)
Number	4	4	5	13	9	
Age (d)	43 ± 7	40 ± 7	37 ± 2	40 ± 5	37 ± 4	
Days after surgery	22 ± 10	6 ± 1	4 ± 2	10 ± 9	29 ± 3	
Body mass (kg)	13.2 ± 2.4	12.0 ± 2.4	13.6 ± 3.7	13.0 ± 2.8	10.5 ± 2.3	<0.05
Total water (mL/kg)	765 ± 22	791 ± 58	784 ± 40	780 ± 40	752 ± 27	
V_{ec} (mL/kg)	277 ± 12	278 ± 19	276 ± 19	277 ± 15	307 ± 27	<0.01
V_{ic} (mL/kg)	488 ± 16	512 ± 49	503 ± 36	501 ± 35	445 ± 27†	<0.001
Blood volume (mL/kg)	78 ± 21	83 ± 34	83 ± 13	82 ± 21	121 ± 29†	<0.01
Red cell volume (mL/kg)	23 ± 6	22 ± 13	23 ± 6	22 ± 8	48 ± 12†	<0.001
V_{ec}/V_{ic}	0.57 ± 0.05	0.54 ± 0.05	0.56 ± 0.05	0.56 ± 0.04	0.69 ± 0.10†	<0.01

* Control group is divided into lambs that underwent surgery (Control/surgery) more than 2 wk before the experiment or approximately 1 wk before the experiment and lambs that only underwent neck vessel catheterization (Control/no surgery). In addition, pooled data for control lambs are shown (4th column) and the results of the unpaired *t* test for all control ($n = 13$) *vs* hypoxemic lambs ($n = 9$). V_{ec}/V_{ic} = Extracellular to intracellular water ratio.

† Hypoxemic lambs ($n = 9$) significantly different from control lambs ($n = 13$) by analysis of variance and multiple contrasts ($p < 0.05$).

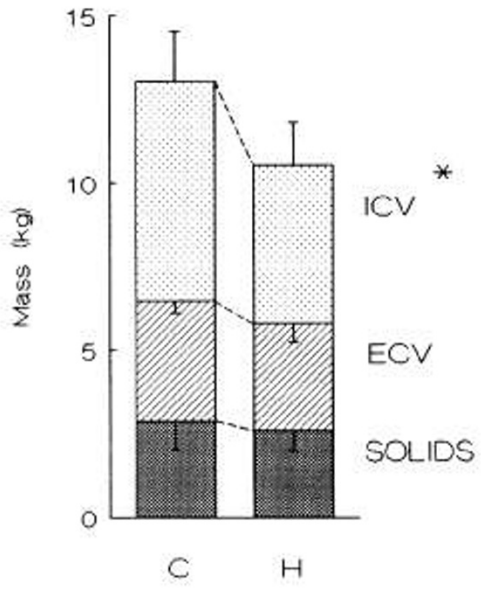

Fig. 1. Body mass in control (*C*) and hypoxemic (*H*) lambs and the division thereof in solids and extracellular (*ECV*) and intracellular (*ICV*) fluid. Mean and SD for solids, ICV, and ECV are shown. *, Hypoxemic lambs ($n = 9$) significantly different from control lambs ($n = 13$) by analysis of variance and multiple contrasts ($p < 0.05$).

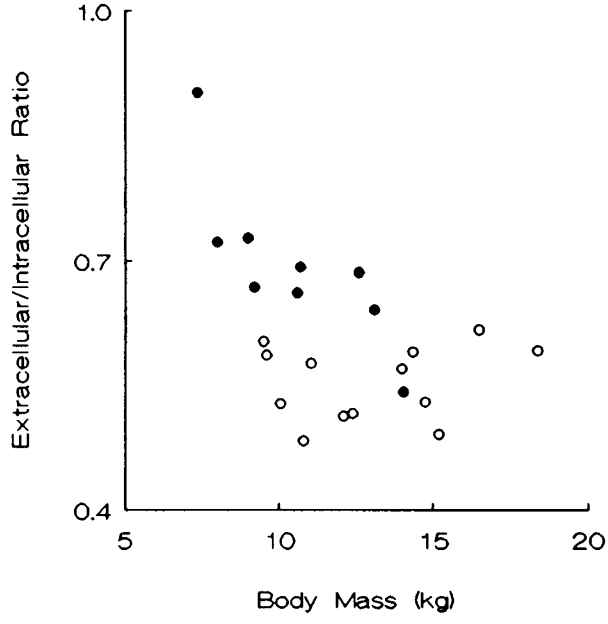

Fig. 2. V_{ec} to V_{ic} ratio (V_{ec}/V_{ic}) as a function of body mass in control (*open circles*) and hypoxemic (*black circles*) lambs.

growth (11). In children, V_{ec} decreases from 350 mL·kg^{-1} to 280 mL·kg^{-1} in the first 6 mo of life, and after 9 to 12 mo V_{ec} makes up a constant fraction of total body water, similar to that in adults (9). Hence, from that time on, the ratio of V_{ec} to V_{ic} (V_{ec}/V_{ic}) will not change anymore. When growth is decreased, one may expect that V_{ec} and V_{ec}/V_{ic} are increased, as compared with those of normally grown peers, and that adult values will be reached later, if at all. This is supported by the observation that, in young rats on a low caloric or a low protein diet, a decreased rate of cellular growth or cellular wasting was accompanied by an increased extracellular volume (26).

Similar changes in body fluid compartment volumes during growth as have been observed in children may be expected in lambs. Presumably, these changes in lambs occur over a shorter period of time, because they grow much faster. Newborn lambs double their body mass in 3 wk and triple it in 7 to 8 wk (27). To the extent that total body water is a good indicator of lean body mass, our results indicate that it makes up a similar fraction of total body mass in hypoxemic and control lambs. However, the fact that V_{ec}/V_{ic} is increased in hypoxemic lambs indicates that intracellular volume in hypoxemic lambs is a smaller fraction of lean body mass than in control lambs. The fact that their $V_{int}/V_{ic}^{extravascular}$, the extravascular equivalent of V_{ec}/V_{ic}, is also increased indicates that the difference in V_{ec}/V_{ic} between hypoxemic and control lambs is not related to the changes in blood volume. In addition, V_{ec}/V_{ic} decreases with body mass in hypoxemic lambs, whereas it is stable over a broad range of body mass in control lambs. This suggests that V_{ec}/V_{ic} in control lambs has become stable and may resemble that of adult sheep, whereas in hypoxemic lambs, it resembles that of younger, less mature lambs. These differences between hypoxemic and control lambs can be explained by assuming a decreased growth of the cellular compartment.

The mechanism of decreased growth in hypoxemia is unclear. In hypoxemic children, no relation between decreased growth and either nutritional intake or the severity of hypoxemia has been found (1–3). In experimental hypoxemic animals, a decreased growth rate has been related to a decreased rate of cell division rather than to a decreased cell volume (4, 5). Both a decreased caloric intake and an effect of hypoxemia on cell division have been implicated as the cause of the decreased rate of cell division (4, 5). The distinction between decreased cell numbers and decreased cell volume is important, because when

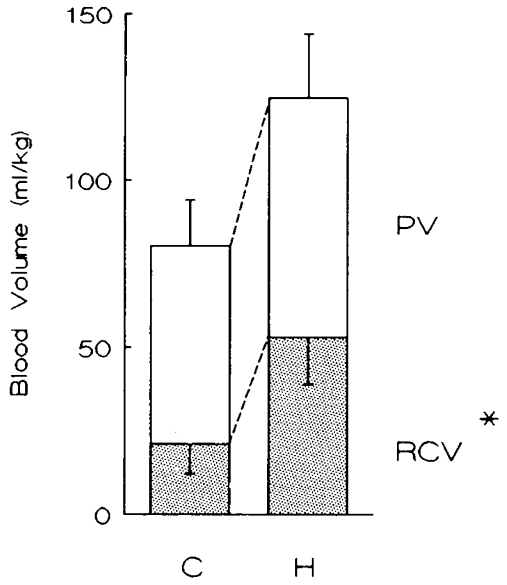

Fig. 3. Blood volume and its components, plasma volume (*PV*), and red cell volume (*RCV*) per unit mass in control (*C*) and hypoxemic (*H*) lambs. Mean and SD for RCV and PV are shown. *, Hypoxemic lambs ($n = 9$) significantly different from control lambs ($n = 13$) by analysis of variance and multiple contrasts ($p < 0.05$).

cell numbers are decreased, catch-up growth may not completely compensate for the growth retardation (28). The results of our study, however, offer no clues about the mechanisms of the decreased cellular growth in hypoxemia.

The second goal of our study was to determine the effects of chronic hypoxemia on blood volume and its components. Blood volume in hypoxemic lambs was increased, mainly through an increased red cell volume. This is consistently found in hypoxemia (12, 13, 29, 30). The effects of hypoxemia on plasma volume, however, are variable. In acute hypoxemia, plasma volume decreases (24, 31). In chronic hypoxemia, plasma volume is normal but tends to decrease when hematocrit increases to over 60% (12, 13, 29). In rats exposed to simulated high altitude, plasma volume decreased concurrent with an increase in both total blood volume and red cell volume, whereas hematocrit increased from 50% at sea level to 75% at high altitude (30). The mechanism behind the decrease in plasma volume is unclear. Inasmuch as the decrease in plasma volume prevents an excessive increase in blood volume, it may be mediated through atrial natriuretic peptide release, as has been shown in conditions of increased intravascular volume (32). A disadvantage of a decrease in plasma volume is a further increase of blood viscosity. Even though blood volume was increased by 50% in our hypoxemic lambs, there were no signs of a decreased plasma volume or of increased vascular filling, suggesting that a mechanism to decrease the intravascular volume was not yet activated.

In conclusion, we have demonstrated that V_{ic} is decreased in chronically hypoxemic lambs and that this is the main cause of their decreased body mass. The alterations in V_{ec} and V_{ic} per unit mass and in V_{ec}/V_{ic} can be explained by assuming a decreased growth of the cellular compartment. They are not related to the increase in blood volume, which is mainly brought about by an increase in the red cell volume. These results confirm and extend previous observations that the adaptation to chronic hypoxemia in lambs is established almost completely through an increase in Hb concentration and at the expense of growth, by linking the decreased body mass to a decreased intracellular volume and by showing the extent of the erythropoietic response.

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REFERENCES

1. Linde LM, Dunn OJ, Schireson R, Rasof B 1967 Growth in children with congenital heart disease. J Pediatr 70:413–419
2. Salzer HR, Haschke F, Wimmer M, Heil M, Schilling R 1989 Growth and nutritional intake of infants with congenital heart disease. Pediatr Cardiol 10:17–23
3. Strangway A, Fowler R, Cunningham K, Hamilton JR 1976 Diet and growth in congenital heart disease. Pediatrics 57:75–86
4. Elliott DA, Cheek DB 1968 Muscle and liver cell growth in rats with hypoxia and reduced nutrition. In: Cheek DB (ed) Human Growth. Lea & Febiger, Philadelphia, pp 326–336
5. Naeye RL 1966 Organ and cellular development in mice growing at simulated high altitude. Lab Invest 15:700–706
6. Rabinovitch M, Gamble W, Nadas AS, Miettinen OS, Reid L 1979 Rat pulmonary circulation after chronic hypoxia: hemodynamic and structural features. Am J Physiol 236:H818–H827
7. Teitel DF, Sidi D, Bernstein D, Heymann MA, Rudolph AM 1985 Chronic hypoxemia in the newborn lamb: cardiovascular, hematopoietic, and growth adaptations. Pediatr Res 19:1004–1010
8. Turek Z, Turek-Maischeider M, Claessens RA, Ringnalda BEM, Kreuzer F 1975 Coronary blood flow in rats native to simulated high altitude and in rats exposed to it later in life. Pflugers Arch 355:49–62
9. Cheek DB, Talbert JL 1968 Extracellular volume (and sodium) and body water in infants. In: Cheek DB (ed) Human Growth. Lea & Febiger, Philadelphia, pp 117–164
10. Fomon SJ, Haschke F, Zierler EE, Nelson SE 1982 Body composition of reference children from birth to age ten years. Am J Clin Nutr 35:1169–1175
11. Cheek DB, Wishart J, MacLennan AH, Haslam R 1984 Cell hydration in the normally grown, the premature and the low weight for gestational age infant. Early Hum Dev 10:75–84
12. Rosenthal A, Button LN, Nathan DG, Miettinen OS, Nadas AS 1971 Blood volume in cyanotic congenital heart disease. Am J Cardiol 27:162–167
13. Verel D 1961 Blood volume changes in cyanotic congenital heart disease and polycythemia rubra vera. Circulation 23:749–753
14. Zweens J, Frankena H, Rispens P, Zijlstra WG 1975 Determination of extracellular fluid volume in the dog with ferrocyanide. Pflugers Arch 357:275–290
15. Zweens J, Frankena H, Zijlstra WG 1980 Infrared-spectrometric determination of D_2O in biological fluids. Reappraisal of the method and application to the measurement of total body water and daily water turnover in the dog. Pflugers Arch 385:71–77
16. Zweens J, Frankena H 1981 An improved method for the determination of the plasma volume with Evans Blue. J Clin Chem Clin Biochem 19:919–924
17. Schrier RW 1988 Pathogenesis of sodium and water retention in high-output and low-output cardiac failure, nephrotic syndrome, cirrhosis, and pregnancy. N Engl J Med 319:1065–1072
18. Villarreal D, Freeman RH, Davis JO, Verburg KM, Vari RC 1987 Atrial natriuretic factor secretion in dogs with experimental high-output heart failure. Am J Physiol 252:H692–H696
19. Gratama JWC, Dalinghaus M, Meuzelaar JJ, Gerding AM, Koers JH, de Graaf MY, Kuipers JRG 1992 Body fluid compartments in lambs with an aorto-pulmonary shunt. J Clin Invest 90:1745–1752
20. Barger AC, Ross RS, Price HL 1955 Reduced sodium excretion in dogs with mild valvular lesions of the heart, and in dogs with congestive heart failure. Am J Physiol 180:249–259
21. Barger AC, Wilson GM, Price HL, Ross RS, Brooks L, Boling EA 1955 Relationship between exchangeable sodium and rate of sodium excretion in dogs with experimental valvular lesions of the heart. Am J Physiol 180:387–391
22. Farber MO, Bright TP, Strawbridge RA, Robertson GL, Manfredi F 1975 Impaired water handling in chronic obstructive lung disease. J Lab Clin Med 85:41–49
23. Sapir DG, Levine DZ, Schwartz WB 1967 The effects of chronic hypoxemia on electrolyte and acid-base equilibrium: an examination of normocapneic hypoxemia and of the influence of hypoxemia on the adaptation to chronic hypercapnia. J Clin Invest 46:369–377
24. Honig A 1989 Peripheral arterial chemoreceptors and reflex control of sodium and water homeostasis. Am J Physiol 257:R1282–R1302
25. Ou LC, Silverstein J, Edwards BR 1984 Renal function in rats chronically exposed to high altitude. Am J Physiol 247:F45–F49
26. Fiorotto ML, Sheng HP, Evans HJ, Leblanc AD, Johnson PC, Nichols BL 1987 Specific effects of weight loss, protein deficiency and energy deprivation

on the water and electrolyte composition of young rats. J Nutr 117:933–940
27. Lister G, Walter TK, Versmold HT, Dallman PR, Rudolph AM 1979 Oxygen delivery in lambs: cardiovascular and hematologic development. Am J Physiol 237:H668–H675
28. Widdowson EM, McCance RA 1975 A review: new thoughts on growth. Pediatr Res 9:154–156
29. Sanchez C, Merino C, Figallo M 1970 Simultaneous measurement of plasma volume and cell mass in polycythemia of high altitude. J Appl Physiol 28:775–778
30. Ou LC, Cai YI, Tenney SM 1985 Responses of blood volume and red cell mass in two strains of rats acclimatized to high altitude. Resp Physiol 62:85–94
31. Grover RF, Reeves JT, Maher JT, McCullough RE, Cruz JC, Denniston JC, Cymerman A 1976 Maintained stroke volume but impaired oxygenation in man at high altitude with supplemental CO_2. Circ Res 38:391–396
32. Goetz KL 1988 Physiology and pathophysiology of atrial peptides. Am J Physiol 254:E1–E15