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Effects of hypothermic perfusion on pulmonary circulation in isolated rabbit lung*

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The effects of hypothermic perfusion have been studied by using different perfusates in 24 isolated rabbit lung preparations, divided into three groups: G1, perfused with blood (hematocrit of 10 %) and G2 and G3, perfused with erythrocyte-free plasma plus 6 % protein in saline. In both G1 and G2 groups left atrial pressures were kept below airway pressure (Zone II conditions), and in G3 it was higher than airway pressure (Zone III conditions). Perfusate flow, pulmonary artery pressure, pulmonary vascular resistance, left atrial pressure, fluid filtration rate, colloid osmotic pressure and temperature were not different ($p > 0.1$) between G1 and G2 at the beginning of the experiments. Lowering perfusate temperature from 38 °C to 28 °C produced a significant increase in pulmonary artery pressure and pulmonary vascular resistance in G1 but they decreased in G2 lungs ($p < 0.05$). Fluid filtration rate increased in both groups during hypothermia. These responses were not inhibited by an α-adrenergic receptor blocker or a pulmonary vasodilator. In G3 lungs no changes were observed. The differences in the hemodynamic effects of hypothermia observed in G1 and G2, both in Zone II conditions, could result from the differences in the vessel distention state obtained by each of the perfusate before initiating hypothermia. As perfusate viscosity increase with cold, a greater possibility of vessel distention in G2 lungs occurs. This explains the decrease in pulmonary artery pressure and pulmonary vascular resistance with cold in this group. The increase in fluid filtration rate observed with hypothermia in G1 and G2 may be due to increases in fluid exchange area.

Key words: Hypothermia, Pulmonary circulation, Pulmonary artery pressure.

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Hypothermia is a therapeutic modality mostly used in critical care, cardiovascular surgery, neurosurgery and anesthesiology (9, 11, 15). In pulmonary circulation it is believed to produce an increase in pulmonary artery pressure (Ppa) and an increase in pulmonary vascular resistance (PVR) (2). Others (5), however, have found no effect of hypothermia on pulmonary vasculature and there are reports that hypothermia may even lower Ppa. Information of its effect on pulmonary water balance is scant and inconsistent. Beneficial and deleterious effects have been reported (11). These different and conflicting results are probably due to the use of *in situ* and *in vivo* experimental models, different perfusates and perfusion conditions. In the present work the effects of cold perfusion in isolated rabbit lung preparations have been studied in which all the relevant hemodynamic parameters, perfusion conditions and type of perfusate could be controlled.

Materials and Methods

These experiments were carried out using an isolated perfused rabbit lung preparation, described previously (12, 18). Briefly, 24 New Zealand rabbits weighing between 2–3 kg were anesthetized with i.p. sodium pentobarbital (30–40 mg/kg). Animals were exsanguinated and the heart and lungs were rapidly removed. Silastic cannulae were inserted into the pulmonary artery and left atrium. The preparation was suspended on a force transducer (Grass Force Displacement Transducer FT03C, Quincy, Mass.) at the top of a Perspex box which was kept at a temperature of 34–36 °C and constant humidity (fig. 1). Ppa, left atrial pressure (Pla) and airway pressure (Paw) were measured with pressure transducers (Gould Statham P23Db, Hato Rey, PR) and displayed on a polygraph (Grass 7B Polygraph, Quincy

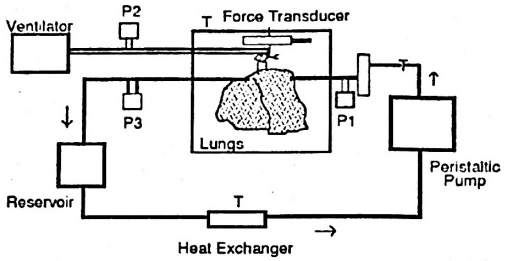

Fig. 1. *Simplified diagram of the isolated lung preparation. Lungs are suspended to a force transducer fixed at the top of an humidified and temperature controlled perspex box.*
Blood, after passing through the heat exchanger, is pumped to the lungs and later collected at the reservoir, which could be varied in height, thus permitting adjustments in left atrial pressure. P1 = pulmonary artery pressure transducer. P2 = airway pressure transducer. P3 = left atrial pressure transducer. T = temperature probe.

Mass.) together with changes in lung weight. Measurements of weight changes of the preparation were used to estimate the fluid filtration rate (FFR) of the lung (9, 11). Although the differentiation between central blood volume changes and FFR may prove difficult from using this method, it is generally accepted that rapid weight changes represent pulmonary blood volume changes, whereas a constant or stable slope in the lung weight curve is probably related to the FFR of the preparation. We used this portion of the lung weight curves to estimate FFR. The zero pressure reference for vascular pressures was the bottom of the lungs. The preparation height was 7–8 cm from apex to lung base. Lungs were perfused with a roller pump (Masterflex Digistaltic Roller Pump, Chicago, Ill) maintaining a constant flow throughout the experiments. Perfusion was started within 10 minutes of exsanguination. Oscillations of the roller pump were dampened by directing the perfusate flow (PF) to an air filled chamber which also served as a bubble trap. The perfusate passed through a long

silastic coil (heat exchanger) which was immersed in a temperature–controlled heating bath before entering the lungs. The bath temperature could be changed from 38 °C to 28 °C in less than 5 minutes. Perfusate temperature was checked by a thermometer probe in the arterial line.

The preparation was perfused by using two different perfusates: 1) blood obtained from the same animal was diluted to a 10 % hematocrit (Htc) with a 0.9 % NaCl + 6 % albumin (blood 10 % Htc perfusate), and 2) 0.9 % NaCl + 6 % albumin solution to which the animal's blood plasma (erythrocyte–free perfusate) was added.

Lungs were ventilated with a small animal ventilator (Harvard Apparatus, Natick, Mass) with a tidal volume of 10 ml/kg. A 5 % CO_2–air mixture was used for ventilating the lungs. Blood gases and pH were measured with a Radiometer BMS3 MK2 Blood Mycro System (Copenhagen, Denmark) which was calibrated using standard gas mixtures. Values of blood gases and pH were corrected to perfusate temperature.

Hematocrit was measured in duplicate samples in an Autocrit II Centrifuge (Clay Adams, Parsippany, NJ). Colloid osmotic pressure (COP) was measured with a Wescor 4400 Colloid Osmometer (Logan, Utah) with a 30,000 MW cut off membrane and calibrated with a water manometer and a standard protein solution.

Data are expressed as mean ± SEM unless stated otherwise. All results were analyzed using Student's *t* test for two populations or for matched samples where applicable. Level of significance was $p < 0.05$.

Experimental Protocol.– Lungs were allowed to stabilize hemodynamically for 30 minutes, until a constant FFR was obtained. Simultaneously acid–base balance was adjusted by adding small amounts of sodium bicarbonate to the blood reservoir. Lungs were divided into three groups: G1 was perfused with the 10% Htc solution, and G2 and G3 with the red blood cell–free perfusate. In G1 and G2 Pla was kept below Paw in order to obtain a preparation mostly in West's Zone II perfusion conditions. In G3, Pla was increased, by elevating the blood reservoir, to a level higher than Paw to obtain a Zone III condition (19).

Perfusate temperature was set at 38 ± 0.1 °C at the beginning of the experiments. A complete set of measurements, including Ppa, Pla, Paw, PF, FFR, Htc, COP, perfusate temperature, pH, PCO_2 and PO_2, was obtained after the stabilization period. Perfusate temperature was then lowered to 28 ± 0.1 °C. After 10 minutes (18) a new hemodynamic and fluid balance equilibrium was obtained and a new set of measurements was taken. Perfusate temperature was then returned to the basal level (38 ± 0.1 °C) and measurements were again obtained after equilibrium.

In some experiments papaverine hydrochloride 70–80 μg/ml (8) or phentolamine 25–27 μg/ml was added to the perfusate (180–200 ml) and repeated the experimental protocol. This study was approved by the "Comité Institucional del Cuidado y Uso de Animales", Campo de Ciencias Médicas, Universidad de Puerto Rico, and the Instituto de Medicina Experimental, UCV, Caracas (Venezuela).

Results

There was no statistically significant difference between the PF of the three lung groups. G1 and G2 lungs were in similar hemodynamic condition at the beginning of the experiments (same level

of PF, Ppa, Pla and PVR). In G1 lungs Ppa, PVR and FFR increased significantly as perfusate temperature was lowered from 38 to 28 °C. Upon rewarming the perfusate to 38 °C these variables returned to the initial values (fig. 2). In G2 lungs FFR also increased significantly with hypothermia but Ppa and PVR showed a significant decrease. As with G1 lungs, these values returned to their previous levels with rewarming (fig. 2). G3 lungs had a higher Pla than Paw, thus creating a Zone III perfusion condition. This group also had a significantly higher initial FFR and a lower PVR than G1 and G2. Hypothermia did not have an effect on Ppa and PVR but there was a significant decrease in FFR after rewarming (fig. 3).

The addition of papaverine hydrochloride or phentolamine to the perfusate did not change the obtained results.

Table I gives the acid–base and COP values of the preparations. As there were no significant differences between the groups they are presented as pooled means. PO_2 and PCO_2 were read at 37 °C and corrected to the perfusate temperature by using temperature–dependent solubility tables. Values of pH, corrected for perfusate temperature, showed a significant increase during hypothermia; this is

Table I. *Acid–base values and colloid-osmotic pressure (COP) of perfusates (G1, G2 and G3 lungs) in experimental protocols (n=24).* Pressure in mmHg. Results are given as Mean ± SEM.

	Perfusate Temperature (°C)		
	38	28[1]	38
pH	7.43±0.03	7.51±0.03	7.37±0.06
PO_2	110.2±17.9	105.5±15.9	11.40±14.6
PCO_2	23.6±2.9	21.5±1.7	23.8±3.2
COP	18.1±0.7	17.9±1.0	18.0±0.8

[1]Values corrected for temperature.
(* $p<0.05$).

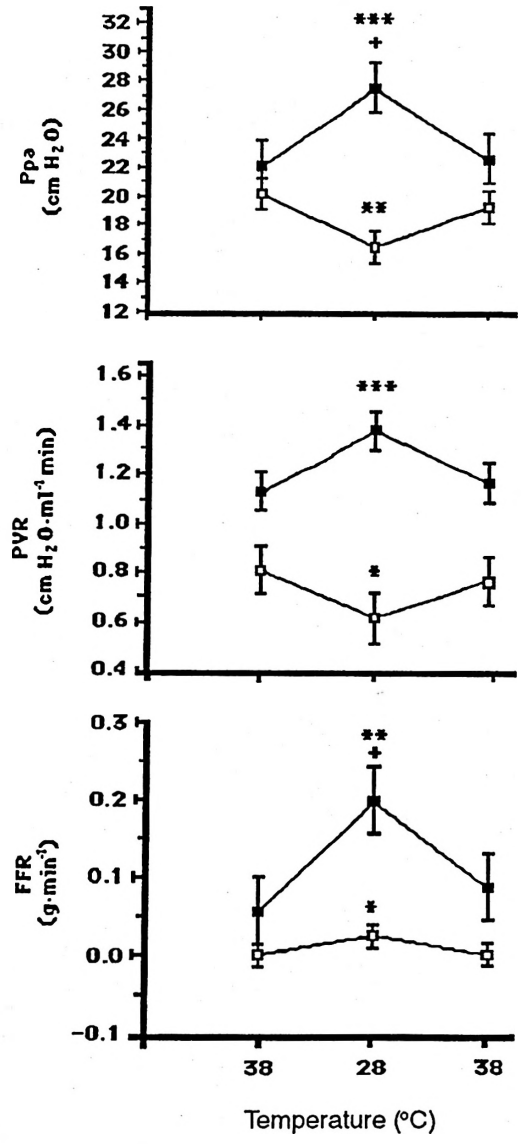

Fig. 2. *Effects of hypothermia on pulmonary artery pressure (Ppa), pulmonary vascular resistance (PVR) and fluid filtration rate (FFR) in G1 (n = 12) and G2 (n = 7), both of which were in Zone II conditions (airway pressure = 8.3 ± 0.9 cm H_2O > left atrial pressure = 0.4 ± 0.7 cm H_2O).*
Solid squares: G1 lungs perfused with blood 10 % hematocrit. Open squares: G2 lungs perfused with the erythrocyte-free perfusate. * $p < 0.05$, ** $p < 0.025$, *** $p < 0.005$ from values measured at 38 °C with the same perfusate; + $p < 0.05$, from corresponding values with different perfusate.

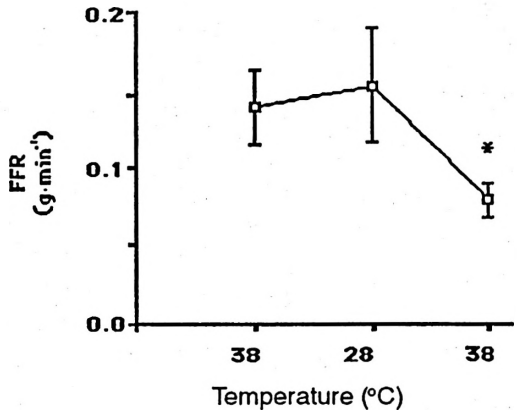

Fig. 3. *Effects of hypothermia on fluid filtration rate (FFR) in G3 lungs (n = 5), which were in Zone III conditions (airway pressure = 7.4 ± 0.9 cm H_2O < left atrial pressure = 9.1 ± 0.9 cm H_2O) and perfused with an erythrocyte-free perfusate. (* $p < 0.05$).*

mainly due to the variation of the neutral pH of water with temperature and should not be interpreted as an alkaline state (13, 17).

No changes were found in COP with hypothermia in G1, G2 and G3. Hematocrit in the G1 lungs was 9.47 ± 0.76 %, with no statistical difference during hypothermia.

Discussion

This study has evaluated the effects of hypothermic perfusion in an isolated rabbit lung preparation. Different hemodynamic responses were found in lungs depending on the perfusate used. In G1 lungs, perfused with blood 10 % Htc, hypothermia increased Ppa and PVR whereas in G2 lungs, perfused with the red blood cell–free perfusate, hypothermia decreased Ppa and PVR. Both groups were in Zone II conditions. In G3 lungs, which were perfused with the red blood cell–free perfusate and in Zone III conditions, hypothermia did not change Ppa or PVR. The higher initial FFR and lower PVR of this group compared with G1 and G2 lungs, may be explained by the increased vascular exchange area in Zone III perfusion conditions.

The reason for selecting 10 % for the Htc of G1 lungs was to have a perfusate with red blood cells but whose slightly higher viscosity in comparison to the red blood cell–free perfusate would not affect the hemodynamic parameters measured.

The response of the pulmonary vasculature to cooling in whole animal preparations is reported to be an increase in Ppa and PVR (2). The mechanism of this response has been attributed to pulmonary vasoconstriction, to increased viscosity or both. In studies of this nature it is important to differentiate between active vasomotion and passive hemodynamic changes. As our studies used isolated preparations, the systemic responses to cold, such as an increased catecholamine outflow (10), cannot be present. Previous authors have reduced the cold–induced increase in Ppa by using α-receptor blockers. On the basis of these results they concluded that part of the pulmonary constriction to cold is mediated through α-receptors of the sympathetic nervous system; others have not found such an effect. The hemodynamic responses to hypothermia could not be abolished in our preparations by blocking pulmonary vascular responses with either the α-receptor blocker phentolamine or by using the pulmonary vasodilator papaverine hydrochloride (8). Therefore, we believe that our results are mainly due to passive hemodynamic changes produced by the cold induced increase in viscosity of the perfusate. The possibility that the changes observed were mediated by changes in Htc or COP were ruled out as these parameters were constant throughout the experiments. Increases in Htc and COP

have been reported with hypothermia (5, 14) when using whole animal models.

One possible explanation for our results, which take into account perfusion conditions, is presented. G1 and G2 lungs were in West's Zone II conditions (19). If pulmonary circulation is viewed as a group of parallel non-distensible Starling resistors (3), one above the other, any increase in perfusate viscosity would produce an increase in Ppa if constant flow is maintained (fig. 4A). This did occur in G1 lungs. The lowering of Ppa in G2 lungs with hypothermia could be explained by assuming that capillary vessels are distended as a result of the increase in viscosity of the perfusate, thus lowering PVR (fig. 4B). We believe that vessels perfused with blood 10 % Htc were already more distended than the ones perfused with the red blood cell–free perfusate, so that the possibility of further distending vessels in G1 lungs was less than in G2. As the perfusate viscosity is increased during the cooling period, the G1 lungs experience an increase in Ppa whereas the G2 lungs, having a greater initial capacity to distend, show a decrease in Ppa and PVR.

To test this hypothesis similar experiments were performed with the G3 group, in which lungs were in Zone III perfusion conditions. As this group had a high Pla it probably had most of its vasculature already distended. In this group hypothermia did not change Ppa or PVR. The added resistance due to the perfusate higher viscosity would be small when geometrical resistance of the system as a

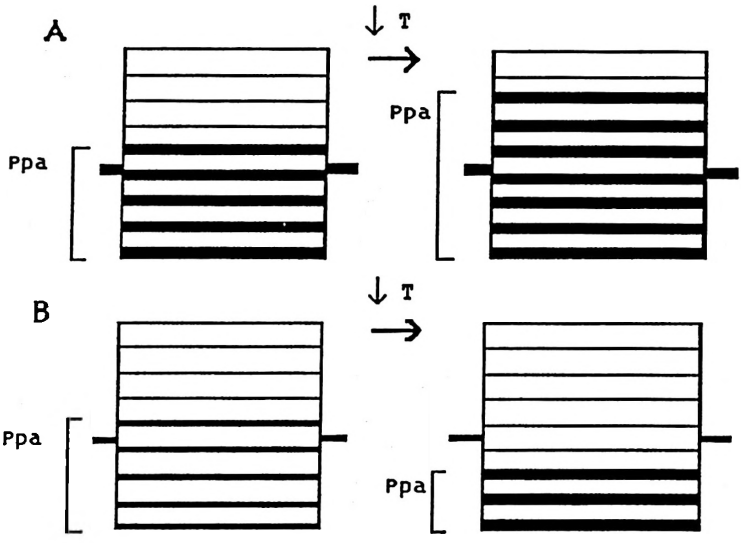

Figure 4. *Pulmonary vascular bed models showing different levels of vessel recruitment and distention.*
A: lungs perfused with 10 % hematocrit. This system has a higher degree of vessel distention at the beginning of experiments. When perfusate temperature is lowered and its viscosity increased, pulmonary artery pressure (Ppa) increases. B: Lungs perfused with the red blood cell free perfusate. This system has the same Ppa than A, but a smaller degree of vessel distention. As viscosity of the perfusate is increased by cold, pulmonary vessels distend and Ppa decreases. (T= temperature).

whole is at a minimum as in Zone III perfusion conditions.

The behavior of Ppa and PVR in our experiments cannot be explained with the Starling resistor model of pulmonary circulation. Newer pulmonary vascular models had to introduce distensible elements in their formulations in order to explain deviations from this model (3, 4, 6, 7, 16). We are also aware of the difficulty in differentiating between pulmonary vascular recruitment versus pulmonary vascular distention as a mechanism involved in our experiments. As viscosity of the perfusate increased with cold, it probably produced a greater level of recruitment in G1 lungs, whereas it increased the degree of vessel distention in G2 lungs.

We think that viscous factors tending to increase resistance will evoke changes in pulmonary vasculature which will increase or decrease resistance, depending on the different level of vessel distention produced by the perfusate. Since the increase in viscosity by a similar degree of cooling between plasma and blood is very similar, especially with blood with 10 % Htc, the role of the decrease of red blood cell flexibility with cold (19) remains to be established.

FFR increased as temperature decreased, being more evident in G1 lungs. Hypothermia has been advocated as an edema protective factor in certain isolated lung preparations. As results were not different when preparations were treated with alpha receptors blockers or with pulmonary vasodilators the increases in FFR with hypothermia may be the result of passive mechanical factors such as an increase in perfusate viscosity. We believe that the increases in FFR in Gl and G2 lungs, during hypothermic perfusion, were due to an increase in vascular exchange area probably related to the cold-induced increase in perfusate viscosity. In G1 the probable mechanism is vessel recruitment whereas in G2 is vessel distention. As noted earlier, G3 lungs had a lower PVR, which may have masked any increases in FFR with hypothermia. Furthermore, G3 lungs had a higher initial FFR because of the increased vascular exchange area of the Zone III perfusion conditions. After rewarming, FFR was lower in this group than at the beginning of the experiment. It could be that the higher fluid transfer to the interstitium elevated interstitial pressure, resulting in a new force balance, opposing the fluid tendency to accumulate in this space.

In summary, in our experiments hypothermia produced passive hemodynamic changes in pulmonary vasculature, which were correlated with the initial distention/recruitment vessels. Hypothermic perfusion may increase lung water because of these hemodynamic changes.

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R. MARTÍNEZ-RUIZ, A. H. SILLAU, M. A. RICO-ORSINI, S. TRISTANO-CASTIGLIONI y R. SÁNCHEZ DE LEÓN. *Efectos de la perfusion hipotérmica pulmonar en pulmón aislado de conejo*. J. Physiol. Biochem. (Rev. esp. Fisiol.), 52 (1), 1-8, 1996.

Se estudia el efecto de una perfusión hipotérmica, en 24 preparaciones de pulmón aislado y perfundido de conejo, usando diferentes líquidos perfusores. Los pulmones se dividen en tres grupos: G1 en donde se perfunde sangre (hematocrito del 10 %); G2 y G3 perfundido con plasma libre de eritrocitos y solución salina con albúmina al 6 %. En ambos grupos G1 y G2 se mantiene la presión de la aurícula izquierda por debajo de la presión de la vía aérea (condiciones de Zona II, West) y en el G3 la presión auricular es mayor que la presión de la vía aérea (condiciones de Zona III.

West). Se mide de manera continua el flujo utilizado, la presión arterial pulmonar, la presión auricular izquierda, la tasa de filtración de líquidos, la temperatura del líquido perfusor y de la caja con la preparación, la presión coloido osmótica al principio y al final de cada experimento, y se calcula en cada fase la resistencia vascular pulmonar. El flujo, las presiones auricular, pulmonar y coloido osmótica, la temperatura, tasa de filtración de líquidos y la resistencia vascular pulmonar no difieren entre G1 y G2 ($p > 0.1$) al comienzo de los experimentos. El descenso de la temperatura del líquido perfusor (38-28 °C) produce una elevación significativa de la presión arterial y de la resistencia vascular en los pulmones del G1 ($p < 0,05$); en los pulmones del G2 se observa un descenso tanto de la presión arterial pulmonar como de la resistencia vascular pulmonar. La tasa de filtración de líquidos aumenta en ambas condiciones. Estas respuestas no se inhiben por la utilización previa de un bloqueador α-adrenérgico ni de un vasodilatador pulmonar. En el G3 no se observan cambios significativos. Se considera que las diferencias hemodinámicas observadas durante la hipotermia entre G1 y G2 (Zona II) podría deberse a diferencias en el grado de distensión obtenido antes de iniciar la hipotermia según el tipo de perfusor utilizado. Cuando aumenta la viscosidad del líquido perfusor, por descenso de la temperatura, se observa una mayor distensión vascular (G2), lo que explica la disminución en la presión arterial pulmonar y en la resistencia vascular pulmonar con la hipotermia en G2, y el aumento de la tasa de filtración de líquidos con la hipotermia en G1 y G2, probablemente por aumento de la superficie de intercambio vascular.

Palabras clave: *Hipotermia, Circulación pulmonar, Presión arterial pulmonar*

References

1. Anderson, D. R., Sweeney, D. J. and Williams, T. A. (1986): Statistics: Concepts and applications. West Publishing Co. St. Paul.
2. Benumof, J. L. and Wahrenbrock, E. A. (1977): *J. Appl. Physiol.*, 42, 56–58.
3. Bshouty, Z. and Younes, M. (1990): *J. Appl. Physiol.*, 68, 1514–1527.
4. Ducas, J., Schick, U., Girling, L. and Prewitt, R. M. (1988): *Am J Physiol.*, 255 (*Circ. Physiol.*, 24), H19–H25.
5. Green, J. F and Jackman, A. P. (1979): *Circ. Res.*, 44, 411–419.
6. Hakim, T. S., Chang, H. K. and Michel, R. P. (1985): *Respir. Physiol.*, 61, 115–123.
7. Hasinoff, I., Ducas, J., Schick, U. and Prewitt, R. M. (1990): *J. Appl. Physiol.*, 68, 462–467.
8. Hauge, A. and Nicolaysen, G. (1980): *Acta Physiol. Scand.*, 109, 325–332.
9. Hickey, P. R. and Andersen, N. P. (1987): *J. Cardiothorac. Anesth.*, 1, 137–155.
10. Johnson, T. S., Young, J. B. and Landsberg, L. (1981): *J. Appl. Physiol.*, 51, 614–620.
11. Johnston, W. E., Vinten-Johansen J., Strickland, R. A., Hogan, P. E. and Bowton, D. L. (1989): *Am. Rev. Respir. Dis.*, 140, 110–117.
12. Martínez-Ruiz, R., Zabner, J., Angeli, S. and Sánchez de León, R. (1989): *Intens. Care Med.*, 15, 155–159.
13. Nattie, E. E. (1990): *J. Appl. Physiol.*, 69, 1201–1207.
14. Nose, H. (1982): *Jpn. J. Physiol.*, 32, 831–842.
15. Siesjo, B. K., Carlsson, C., Hagerdal, M. and Nordstrom, C. H. (1976): *Crit. Care Med.*, 4, 283–294.
16. Soohoo, S. L., Goldberg, H. S., Graham, R. and Jasper, A. C. (1987): *J. Appl. Physiol.*, 62, 1982–1988.
17. Swain, J. A. (1988): *Arch. Intern. Med.*, 148, 1643–1646.
18. Zabner, J., Angeli, L. S., Martínez-Ruiz, R. and Sánchez de León, R. (1990): *Intens. Care Med.*, 16, 89–94.
19. West, J. B. (1990): Respiratory Physiology: the essentials. (4th ed.). Williams and Wilkins. Baltimore.