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Pulmonary Diffusing Capacity in Lambs during the Early Neonatal Period

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Summary

A rebreathing method was used to make 53 measurements of pulmonary diffusing capacity (D_{Lco}) and functional residual capacity (FRC) in 17 newborn lambs during the first 2 days of life. D_{Lco}, FRC, and D_{Lco}/FRC all increased in studies made at 24–48 hr of age compared to those at 2–4 hr of age: D_{Lco} 0.86 ± 0.18 to 1.52 ± 0.09 ml/min/torr; FRC 37 ± 10 to 60 ± 8 ml and D_{Lco}/FRC 2.52 ± 0.75 to 2.89 ± 0.37 ml/min/torr/ml × 10^{-2}. D_{Lco} measured using 0.005% CO in the test gas was not different than that measured using 0.5% CO.

Speculation

The increase in pulmonary diffusing capacity is in part due to an increase in lung volume, but the significant increase in D_{Lco}/FRC demonstrates that other factors such as a change in pulmonary capillary blood volume may also be important. We conclude that there is no evidence for a CO carrier in the early neonatal period in lambs.

The D_{Lco} is an index of the lung's capability for oxygen transport and is dependent upon alveolar volume and pulmonary capillary blood volume. Although D_{Lco} has been measured in human neonates (6, 9, 12), none of these studies have made a systematic examination of the first 2 days of life. The early neonatal period involves the establishment of FRC (1, 7, 8) and the reabsorption of pulmonary fluid (2, 5, 11), which may in turn affect the volume of blood in the pulmonary capillary bed. We have measured D_{Lco} and FRC by a rebreathing method in lambs in lambs at 2–4 hr of age and at 24–48 hr of age in order to evaluate any changes in these parameters.

It has recently been suggested that CO uptake by the lung is not only simple diffusion but in part involves a facilitated transport mechanism (3). In newborn lambs the facilitated component for CO was not present at birth but could be found in lambs at 9 days of age. In an effort to explore the possibility of carrier-mediated CO diffusion, we have made measurements of D_{Lco} at both low (0.005%) and high (0.5%) inspiratory concentrations of CO. We reasoned that if there is any carrier mechanism, D_{Lco} at the high CO levels would be less than that measured at the low level.

MATERIALS AND METHODS

The studies were performed in lambs from mixed Western ewes with known breeding dates delivered at 135–144 days of gestation (term is 147–150 days). Under spinal anesthesia (tetracaine hydrochloride 30–40 mg) cesarean section was performed and the lambs were removed, dried, and placed under a warming lamp. No recuscitative measures were carried out and the lambs in these studies breathed spontaneously with no signs of respiratory distress. Prior to measuring the diffusing capacity, the lambs were lightly anesthetized with phenobarbital (30–50 mg/kg iv) and when quiet they were intubated. Lambs in which repeated studies were made were bottle fed.

D_{Lco} was measured by the rebreathing method (10) with slight modifications for small animals. The volume of the rebreathing system was 400 ml and the lambs were ventilated from a 60-ml syringe. The rebreathing circuit was made up of 3/16-inch ID plastic tubing (Tygon tubing, Norton Plastics and Synthetics Division, Akron, OH) which connected the endotracheal tube and ventilation syringe to a double headed pump (model no. W21R031, Cole Palmer Instrument Company, Chicago, IL) where the gas sample was split and run through a helium analyzer (Warren E. Collins Inc., Braintree, MA) and carbon monoxide analyzer (model no. 865 infrared analyzer, Beckman Instruments Inc., Fullerton, CA) in parallel. The output of both analyzers was continuously recorded (model B44 polygraph, Argonaut Associates Inc., Beaverton, OR) at a paper speed of 1.3 cm/sec. The entire rebreathing circuit was equilibrated with gas made up of either 0.005 or 0.5% CO, 8.0% helium, 20.9% oxygen, and balance nitrogen (Airco Industrial Gases, Vancouver, WA). The circuit was then attached to the endotracheal tube at the end of expiration and the lamb rapidly rebreathed at 40–50 breaths/min with a tidal volume of 60 ml for 30–45 sec.

D_{Lco} and FRC were calculated from the recorded changes in CO and helium concentrations (Fig. 1) as follows

$$D_{Lco} = (0.693/t_{1/2}) \times V_S/BP - 47$$

where D_{Lco} = lung diffusing capacity in milliliters per min per torr STPD; $t_{1/2}$ = time in minutes for [CO] to fall by 50%; V_S = volume of the entire rebreathing system (rebreathing circuit plus lung volume) in milliliters STPD; BP = barometric pressure in torrs.

The half-time for CO was calculated by plotting the drop in CO concentration, after the initial fall due to dilution in the lung volume, on semilog paper *vs.* time. The volume of the rebreathing system was calculated from

$$V_S = V_C \times 8.0/[He]$$

where Vc = the volume of the rebreathing circuit (400 ml) and [He] = the helium concentration measured after dilution into the lung volume. FRC was obtained from V_S-Vc, and is in milliliters STPD. After each measurement the rebreathing circuit was equilibrated with room air and then reconnected to the endotracheal tube. The lamb was then ventilated for 30–45 sec exactly as in the measurement of D_{Lco}. The equilibrium value of CO in this rebreathing with air was used as the back pressure level for pulmonary blood CO and subtracted from the alveolar CO obtained during the initial measurement. The lambs were always studied with 0.005% CO first and this was followed with the 0.5% CO study in an effort to keep blood P_{co} low. The CO analyzer had a built-in linearizer in the 0.5% CO range and we verified that the output was linear in this range of CO by injecting known concentrations of CO into the analyzer. In preliminary studies we performed four consecutive measurements, using the same CO concentration, within a 1-hr period on the same animal. The SD was 6–7.5% of the mean value in these repeated measurements.

RESULTS

The change in concentration of CO and He during a rebreathing experiment are shown in Figure 1, *right panel*. After the initial dilution into lung volume, the uptake of CO follows a single exponential as seen in Figure 1, *left panel*. The birth weight and gestational age at birth of the lambs, the postnatal age at the time D_{Lco} was measured, the CO in the test gas mixture, FRC, $t_{1/2}$, and D_{Lco}/FRC are given in Table 1.

In order to compare lambs over the early neonatal period we have grouped the studies made at 2–4 hr of age *vs.* those made at 24–48 hr of age. In this grouping we took the average D_{Lco} of the measurements made with different CO concentrations in the test gas mixture. We believe this is justified since there appears to be little difference between measurements made with 0.005% CO compared to those with 0.5% CO (see below). D_{Lco} at 24–48 hr was 1.52 ± 0.09 ml/min/torr (mean ± SEM, n = 9), which is significantly greater than the value at 2–4 hr of age; 0.86 ± 0.18 (n = 12), $P < 0.01$, t-test for unpaired samples. If we include only the five lambs who had serial studies, D_{Lco} at 24–26 hr is 1.61 ± 0.09 compared to 0.98 ± 0.06 ($P < 0.01$, t-test for paired samples). There was an increase in FRC from 37 ± 10 to 60 ± 8 but the difference is not significant. Since D_{Lco} is in part dependent on lung volume (9), we have compared D_{Lco}/FRC in the two age groupings of these lambs. At 2–4 hr of age, D_{Lco}/FRC (2.52 ± 0.75 × 10^{-2}) is less than at 24–48 hr (2.89 ± 0.37 × 10^{-2}), $P < 0.05 > 0.025$. Thus, irrespective of any change in lung volume, the diffusing capacity in the newborn lamb lung increases in the early neonatal period.

The effect of varying the concentration of CO at which diffusing capacity was measured was examined in the lambs at 2–4 hr and 24–48 hr of age. In the former animals D_{Lco}/FRC measured with 0.005% CO was greater than that measured using 0.5% CO in the test gas, 2.56 ± 0.25 × 10^{-2} ml/min/torr/ml *vs.* 2.33 ± 0.21 (n = 15). The difference is significant $P < 0.05 > 0.025$ using the t-test for paired samples. However, in the lambs tested at 24–48 hr of age, no difference could be demonstrated: 0.005% = 3.01 ± 0.45 × 10^{-2} *vs.* 0.5% = 2.82 ± 0.32 × 10^{-2} ($P > 0.05$). We conclude that in the first 2 days of life no evidence for the partial saturation of a CO carrier in the lung can be shown.

DISCUSSION

The values obtained for D_{Lco} (Table 1) are similar to those reported by Burns *et al.* (3) in nine newborn lambs (1.46 ± 0.24 ml/min/torr) using the same rebreathing method which we have employed. Our average value at 24–48 hr of age 1.52 ± 0.09) falls within the ranges reported in human newborns at this age (6, 9, 12). These latter studies were all made using a steady state CO uptake technique (4).

The most interesting result of this study is the imcrease in pulmonary diffusing capacity which takes place in the first day of life. Although we observed a 77% increase in the absolute value of D_{Lco} the increase in FRC contributed to this change. Nevertheless, D_{Lco}/FRC increased by 15% from 2–4 hr of age as compared to measurements made in lambs 24–48 hr of age. In an attempt to eliminate the contribution of lung volume to the increase in D_{Lco} we have divided D_{Lco} by FRC. This approach is not entirely justified as it would seem to overemphasize the effects of an increase in FRC. The relationship between D_{Lco} and FRC in our studies is D_{Lco} (milliliters per min per torr) = 0.493 + 0.0135 × FRC (ml). This equation is obtained from all 53 measurements in Table 1 and the relationship is significant, $r = 0.63$, $P < 0.01$. It can be seen from the above relationship that our observed increase in FRC (37 to 60 ml) at 24–48 hr, should produce only a 31% increase in D_{Lco}. Since the actual increase in D_{Lco} (77%) is much greater we conclude that other factors such as pulmonary capillary blood volume must also be involved.

The increase in FRC which we have observed in newborn lambs at 24 hr of age compared to 2 hr seems to be at variance with studies in the human (1, 7, 8). However, the earlier human studies used a plethysmographic technique which measures total thoracic gas volume not FRC. In the study (8) in which helium rebreathing, similar to the method we have used in lambs was employed, only one measurement in each infant was made in the first day of life. This measurement made at about 6–8 hr of age was similar to the determination at 5–6 days. Thus, a strict comparison cannot be made. Inspection of our results in lambs (Table 1) shows that the increase in FRC may have occurred at 4–10 hr of age. However, because most of our studies were carried out either at 2 hr or at 24 hr of age, we cannot state how early in the neonatal period the increase in FRC comes about. The existence of a species difference is also a possible explanation for the inconsistency in the FRC changes.

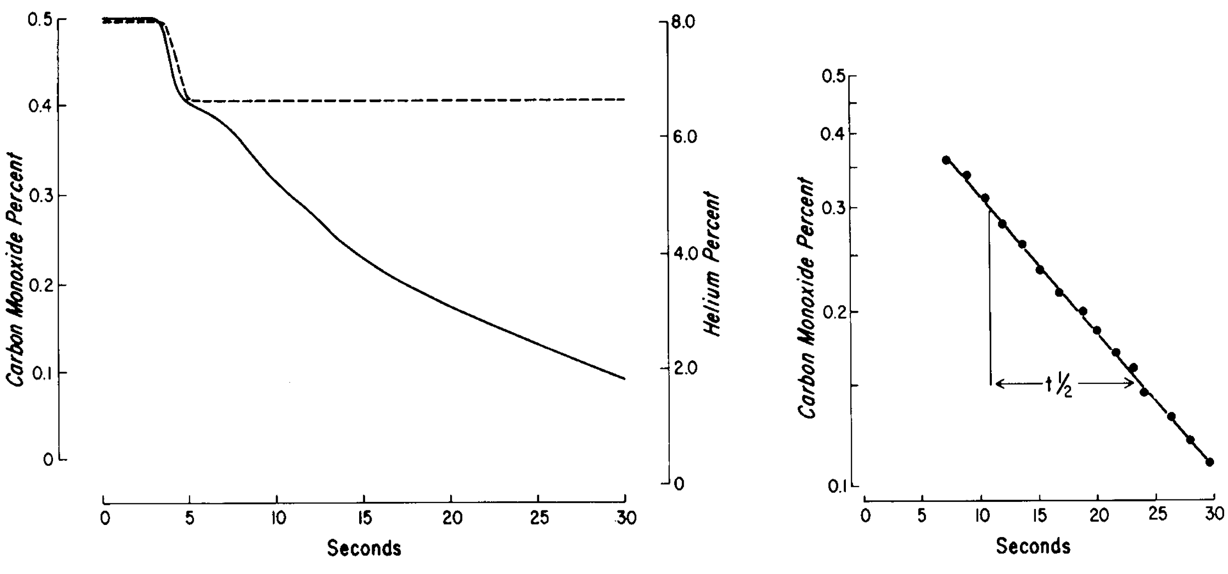

Fig. 1. *Right:* redrawn from a record showing change in CO concentration (——) and helium concentration (- - -) after initiation of rebreathing. Record has not been corrected for instrument response time. *Left:* example of calculation of half-time for CO uptake. Data points taken from the record on the right and CO concentration plotted on a log scale against time in seconds.

Table 1. *Pulmonary diffusing capacities in neonatal lambs*[1]

Birth wt (g)	Gestational age (days)	Postnatal Age (hr)	CO (%)	FRC (ml STPD)	$t_{1/2}$ (sec)	D_{Lco} (ml/min/torr)	D_{Lco}/FRC (ml/min/torr/ml × 10^2)
4147	140	2	0.005	30	26.5	0.69	2.28
		2	0.5	31	34.2	0.60	1.93
3900	140	2	0.005	44	27.9	0.68	1.54
		2	0.05	45	27.8	0.67	1.48
		4	0.005	46	16.9	1.14	2.50
		4	0.5	44	17.0	1.12	2.53
3006	139	2	0.005	27	33.3	0.54	2.02
		2	0.5	34	24.6	0.71	2.07
2400	135	48	0.005	33	12.8	1.35	4.15
		48	0.5	38	18.5	0.95	2.53
		48	0.5	37	17.2	1.02	2.75
2100	144	30	0.005	31	14.5	1.27	4.14
		30	0.5	29	14.3	1.28	4.40
2400	144	30	0.005	29	12.0	1.62	5.65
		30	0.5	31	12.7	1.40	4.49
3100	139	2	0.005	27	16.4	1.10	4.15
		2	0.5	32	15.7	1.17	3.65
		4	0.005	57	27.2	0.73	1.27
3000	139	2	0.005	56	21.0	0.89	1.58
		2	0.5	58	20.1	1.00	1.72
		4	0.005	58	30.8	0.65	1.11
		4	0.5	58	23.5	0.81	1.39
2480	135	2	0.015	40	28.7	0.71	1.79
		2	0.015	42	25.0	0.82	1.94
		2.5	0.1	34	25.0	0.80	2.34
		2.5	0.1	37	26.5	0.74	2.02
3500	142	10	0.005	53	11.5	1.72	3.23
		10	0.5	57	11.3	1.76	3.08
4200	147	48	0.015	53	9.6	1.63	3.10
		48	0.1	62	9.6	1.80	2.93
2115	143	2.8	0.005	23	31.3	0.72	3.13
		2.8	0.5	31	38.3	0.60	1.94
2978	143	2	0.005	27	29.1	0.79	2.93
		2	0.5	27	34.8	0.66	2.44
		2	0.5	31	26.9	0.86	2.77
		24	0.005	74	16.3	1.56	2.11
		24	0.5	90	16.6	1.60	1.78
3824	142	2	0.005	23	21.3	1.06	4.61
		2	0.5	31	18.2	1.27	4.10
		24	0.005	90	16.8	1.59	1.76
		24	0.5	79	13.0	2.00	2.53
2811	142	2.6	0.005	23	29.1	0.78	3.39
		2.6	0.5	31	20.8	1.11	3.58
		24	0.005	74	16.8	1.53	2.07
		24	0.5	64	15.9	1.58	2.47
3000	140	3.3	0.005	31	26.3	0.88	2.84
		3.3	0.5	49	31.2	0.78	1.59
		26	0.005	59	22.1	1.12	1.90
		26	0.5	64	20.7	1.22	1.91
3500	140	3.7	0.005	54	21.2	1.16	2.15
		3.7	0.5	49	20.1	1.21	2.47
		26	0.005	84	14.2	1.87	2.23
		26	0.5	90	13.3	2.01	2.23

[1] CO (%): concentration of CO in the rebreathing gas mixture; FRC: functional residual capacity; t½, time for CO concentration to fall by 50%; D_{Lco}: pulmonary diffusing capacity; D_{Lco}/FRC: pulmonary diffusing capacity normalized with respect to FRC.

Burns *et al.* (3) have presented two arguments in support of the hypothesis that carbon monoxide uptake in the lamb lung is in part carrier mediated. They have shown in lambs at 9 days of age that the administration of 2-diethylaminoethyl-2,2-diphenylvalerate-Hcl (SKF-525A) causes a 45% drop in D_{Lco}. This compound, SKF-525A, binds to cytochrome P-450, the proposed carrier for CO and O_2 in the lung. In contrast, SKF-525A showed no effect on D_{Lco} in newborn lambs (the exact ages are not given). In addition, these authors have shown that D_{Lco} measured with 0.0065% CO in the test gas is the same as that measured with 0.06% CO in newborn lambs. Unfortunately, the studies aimed at demonstrating saturation of a CO carrier were not performed in lambs at age 9 days (3).

We have chosen to measure D_{Lco} at two different CO concentrations in the test gas in an effort to evaluate any saturation of a CO carrier in newborn lambs. In the lambs studied at 2–4 hr of age we found that D_{Lco}/FRC measured with 0.005% CO was 10% greater than that measured using 0.5% CO. While this difference is significant using a paired analysis, the studies at 24–48 hr of age show no difference in D_{Lco} at the two CO concentrations. We conclude that in the first two days of life there is no evidence for partial saturation of a carbon monoxide carrier in the lung.

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