Summary. Pulmonary hyaline membranes developed in guinea pigs exposed for several days to 95% oxygen at atmospheric pressures. This process was accompanied by increased antiplasmin activity in plasma and decreased pulmonary plasminogen activator activity. The latter phenomena may promote the formation of alveolar hyaline membranes by interfering with enzymatic removal of fibrin derived from pulmonary exudation.

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1. Gitlin, D., Craig, J. M., Pediatrics, 1956, v17, 64.

2. Van Breemen, V. L., Neustein, H. B., Burns, P. D., Am. J. Path., 1957, v33, 769.

3. Phillips, L. L., Skrodelis, V., Pediatrics, 1958, v22, 715.

4. Quie, P. G., Wannamaker, L. W., Am. J. Dis. Child., 1960, v100, 836.

5. Samartzis, E. A., Cook, C. D., Acta Pediat., 1960, v49, 724.

6. Samartzis, E. A., Cook, C. D., Rudolph, A. J., ibid., 1960, v49, 727.

7. Ambrus, C. M., Weintraub, D. H., Ambrus, J. L., Pediatrics, in press.

8. Ambrus, C. M., Weintraub, D. H., Niswander, K. R., Ambrus, J. L., ibid., 1965, v35, 91.

9. Ambrus, C. M., Weintraub, D. H., Dunphy, D., Dowd, J. E., Pickren, J. W., Niswander, K. R., Ambrus, J. L., ibid., 1963, v32, 10.

10. Lieberman, J., New Engl. J. Med., 1959, v260, 619.

11. Ambrus, C. M., Fed. Proc., 1966, v25, 68.

12. Ambrus, J. L., Ambrus, C. M., Sokal, J. E., Markus, G., Back, N., Stutzman, L., Razis, D., Ross, C. A., Smith, B. H., Rekate, A. C., Collins, G. L., Kline, D. L., Fishman, J. B., Am. J. Cardiol., 1960, v6, 462.

13. Ploug, J., Kjeldgaard, N. O., Biochim. et Biophys. acta, 1957, v24, 278.

14. Lieberman, J., N. Engl. J. Med., 1961, v265, 363.

15. Mattii, R., Ambrus, J. L., Sokal, J. E., Mink, I., Proc. Soc. Exp. Biol. & Med., 1964, v116, 69.

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Ventilation of Newborn Lambs by Means of a Donor Lung.* (32002)

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Isolated lungs of dogs and cats have been shown to retain their diffusion capacity for many hours(1,2), and excised dog lungs have also displayed a fair ability to function as human kidney(3). In view of the durability and adaptability of pulmonary tissue, an investigation was performed in which donor lungs were attached to the circulation of live animals, and the effects of this shunt upon blood oxygenation and upon survival of the recipient animals were studied.

Although time and circumstances did not permit additional experiments for resolution of the problems involved in this procedure, this report is presented in the belief that the findings may be of use to other investigators working in this field.

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Experimental procedures. Mongrel laboratory dogs were used to supply the donor lungs. The animals were anesthetized (pentobarbital 30 mg/kg i.v.), heparinized (10 mg/100 ml blood), and exsanguinated; the chest was opened through a midsternum incision. The thoracic aorta was ligated in several places in order to block fluid outflow from the bronchial arteries; ligatures were also placed on the superior and inferior venae cavae, and on the azygos vein. The pulmonary artery was cannulated *via* the right ventricle, and a second cannula was placed in the left atrium. Polyethylene tubes were attached to the cannulae, the chest walls were sewn together with tubes emerging, and 4 to 6 liters of a 6% dextran-saline perfusion solution were pumped at low flow rate and pressure (less than 20 mm Hg) *via* the tubes through the lungs for several hours in order

TABLE I. O_2 and CO_2 Levels in Umbilical Blood Before and During Circulation Through the Donor Lung.

Exp. No.	Procedure	Duration of perfusion (min)	Vol % of O_2			Vol % of CO_2		
			Umb. vein (concentration)	Umb. art. (concentration)	Umb. A—V diff.	Umb. vein (concentration)	Umb. art. (concentration)	Umb. A—V diff.
1	Perfusion via donor lung	not recorded	20.2	1.6	18.6	11.0	38.3	27.3
2	*Idem*	" "	11.6	5.2	6.4	22.9	44.2	21.3
3	"	" "	12.5	10.6	1.9	51.1	52.1	1.0
4	Intact		8.9	4.0	4.9	52.5	53.1	0.6
	Cannulated		12.0	10.5	1.5	31.1	47.8	16.7
	Perfusion via donor lung	1	10.5	8.1	2.4	28.8	46.6	17.8
	Idem	10	10.3	7.9	2.4	28.7	45.1	16.4
5	Perfusion via donor lung	15	9.8	7.3	2.5	40.8	50.8	10.0
	Idem	20	10.2	7.8	2.4	42.4	50.5	8.1
	"	35	—	7.9	—	—	49.6	—
	"	40	12.0	7.1	4.9	39.8	47.4	7.6
	"	50	11.8	7.3	4.5	28.8	48.3	19.5
	"	60	11.0	5.2	5.8	34.1	61.5	27.4
6	Intact		14.7	2.4	12.3	36.1	51.4	15.3
	Cannulated		8.1	4.3	3.8	22.8	38.7	15.9
	Perfusion via donor lung	1	7.6	3.9	3.7	22.3	31.5	9.2
	Idem	26	6.8	5.6	1.2	21.4	32.9	11.5
	"	41	6.6	5.8	0.8	23.0	31.5	8.5
*Avg	Intact		11.8	3.2	8.6	44.3	52.2	7.9
	Cannulated		10.0	7.4	2.6	27.0	43.2	16.2
	Perfusion via donor lung		10.8	6.5	4.3	30.4	45.0	14.6

* The author realizes that these averages are not statistically significant; however, they are included as a matter of interest.

to wash out the residual blood and other antigenic substances. Then the pulmonary blood vessels were left filled with the heparinized perfusion fluid at a pressure intermediate between umbilical arterial (70 mm Hg) and umbilical venous (10 mm Hg)(4). The trachea of the dog was intubated, and the tube connected to a respirometer for later positive-pressure respiration either with air or with O_2.

Lambs delivered near term by means of cesarean section, were used as recipient animals. Perfusion *via* the donor lungs was begun within one hour of delivery. Their umbilical veins and arteries were cannulated with polyethylene tubes filled with heparinized perfusion fluid; these tubes were connected to the tubes of the washed donor lung. Immediately, ventilation of the donor lung at the rate of about 10 per minute and tidal volume of about 400 ml was begun, the shunt was opened, and either the umbilical blood was permitted to flow freely through the donor lung or the flow was assisted by gentle pumping. The circulatory shunt thus established had the following course: lamb's umbilical arteries, tubing, donor pulmonary artery, donor pulmonary capillaries, donor pulmonary veins, tubing, lamb's umbilical veins. Simultaneous samples of blood flowing from the lamb to the donor lung and of blood returning from the donor lung to the lamb were taken and analyzed on Van Slyke instruments for O_2 and CO_2 concentrations. In each of the first 3 experiments only one pair of blood samples was taken; in the subsequent 3 experiments up to 5 pairs were taken.

Results. Values of O_2 and CO_2 concentrations in blood are given in Table I. In all cases blood that had passed through the donor lung had acquired O_2 and lost CO_2. The O_2 A-V difference caused by ventilation in the donor lung averaged 4 vol%. The highest O_2 A-V difference, found in Experiment 1, was 18.6 vol%. In this case the inflowing blood had an extremely low O_2 concentration (1.6 vol%). The outflowing blood had an O_2 concentration of 20.2 vol%; this probably matched the O_2 capacity and demonstrated that a well-preserved donor lung was able to function perfectly. The lowest O_2 A-V difference (0.8 vol%) was observed in Experiment

14 after 41 minutes of shunt circulation. It was probably caused by deterioration of the donor lung as well as by dilution of blood with the perfusion fluid.

The CO_2 concentration decreased markedly after blood had passed the donor lung; the average CO_2 A-V difference was 14.3 vol%. This finding suggested the extreme ease with which CO_2 could be eliminated even through a partially deteriorated lung, but it also indicated that the lambs were thrown into a state of severe hypocapnia: The resting metabolism does not call for such a high rate of CO_2 elimination.

The durability of the donor lungs in this study was not as good as had been expected. Despite the fact that the perfusion solution was isosmolar to blood and that the perfusion pressure was kept below the normal pulmonary blood pressure of 20 mm Hg, noises typical of edema were heard in the donor lungs after approximately one hour of perfusion, and, later, fluid appeared in the tracheal tubing. Only one dog showed no signs of pulmonary edema. If the lung was only slightly edematous, ventilation with room air was sufficient to maintain ventilation of blood. With progressing edema, it became necessary to ventilate the lung with pure O_2. The ability of the lung to maintain pressure also deteriorated with progressing edema, and consequently aliquots of the perfusion fluid had to be added periodically to the shunt circulation.

The lambs' blood was perfused *via* the donor lungs for periods of one-half to one hour, and they were free to breathe through their own lungs during this time. All the lambs in this group survived. However, all showed signs of general deterioration during perfusion. They became inactive and some fell asleep or lapsed into coma; in most of them, spontaneous respiration disappeared. Upon disconnection from the donor lungs they recovered, became active, and made attempts to walk which were soon successful. Subsequently, their food intake was normal. They were kept in the laboratory for a week, after which time they were considered permanent survivors.

Discussion. The results of this pilot study suggest that the general concept of temporarily assisting a malfunctioning lung of a newborn individual with a biological donor lung is worthy of further investigation. The deterioration of the lambs during donor lung ventilation was undoubtedly caused primarily by hypocapnia and by hemorrhage into the deteriorating donor lung. Both factors can be controlled to a much better degree than was done in this study.

Hypocapnia could be reduced or even entirely prevented by adding CO_2 to the ventilating gas mixture and by adjusting the rate of blood flow through the donor lung according to both the O_2 and the CO_2 concentrations in the outflowing blood. The blood flow in the intact umbilical circuit of the lamb was measured by Barclay *et al*(4), who report values of 400 ml/min $\pm$ 200.

Our observation of the general behavior of the newborn lambs (and of other newborn animals) and the few values of blood CO_2 concentration obtained prior to shunt circulation suggest that the neonatal respiration normally undergoes large fluctuations caused by fluctuations in motor activity. Newborn animals display alternating phases of vigorous motor activity and complete inactivity. With every spurt of motor activity—struggling, attempting to stand—overventilation seems to occur; this is followed by a collapse, as if in total exhaustion, with prostration on the ground and cessation of breathing. A larger number of samples of CO_2 blood concentration in the first few hours of life would determine whether there is a periodically recurring hypocapnia.

Deterioration of the donor lung could be reduced both by using negative-pressure ventilation(1) and by lowering the pressure of the inflowing perfusion fluid and of the blood by placing resistance in front of the lungs as described by Rosenberg(2).

Even though the bronchial circulatory system in dog(5) is not as complex as that in man(6,7), it is continuous with the pulmonary circuit. Therefore the ligatures which were placed on the large blood vessels in this study (according to the procedure used in conventional laboratory heart-lung preparations) did not necessarily block all channels

of escape from the lung. The perfusion fluid and later the blood could have drained *via* the bronchial arteries and veins into the distal ends of the intercostal arteries and the azygos and the hemiazygos veins respectively and from there into the capillaries of body tissues. If additional ligatures were applied to these vessels, the donor lung should be able to maintain pressure for a longer period of time, and hemorrhage of the recipient animal through the donor lung into the donor animal should be minimized.

There was no indication in this study that immunological reactions played a significant role since the lambs recovered soon after they had been disconnected from the donor lungs and the regulation of respiration was returned to their own respiratory centers. Kimoto *et al*(3), who used dog lungs for dialysis of human blood, give a list of diseases which preclude the utilization of dogs afflicted with them, and they also list several drugs that can be used for disinfection of seemingly healthy dog lungs. With these precautions taken, they feel that the donor lung specimen "could be used without any harm to a human." Nevertheless, further tests for the development of immunological safety are in order. It seems at present that a carefully developed biological donor lung might offer two distinct advantages for temporary assistance of a newborn with a malfunctioning lung: It is a compact structure that is easy to operate, and it is relatively inexpensive.

1. Niden, A. H., Mittman, C., Burrows, B., J. Appl. Physiol., 1962, v17, 885.

2. Rosenberg, E., ibid., 1963, v18, 269.

3. Kimoto, S., Inou, T., Ishii, J.-i., Trans. Am. Soc. Artif. Intern. Organs, 1959, v5, 15.

4. Barclay, A. E., Franklin, K. J., Prichard, M. M. L., The Foetal Circulation, Blackwell Scientific Pub., Oxford, 1944.

5. Miller, M. E., Christensen, G. C., Evans, H. E., Anatomy of the Dog, W. B. Saunders Co., Philadelphia, 1964.

6. Benninghoff, A., Lehrbuch der Anatomie des Menschen, Urban & Schwarzenberg, Munich & Berlin, 3rd ed., 1948.

7. Benninghoff, A., Goerttler, K., ibid., 7th ed., 1964.

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Intestinal Transport of Calcium and Phosphate in Experimental Magnesium Deficiency.* (32003)

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Alcock and MacIntyre(1) reported that in magnesium deficient rats calcium absorption was increased in the absence of magnesium, while magnesium absorption was increased if calcium was absent from the diet. They advanced the hypothesis that magnesium and calcium are absorbed by a common transport mechanism. An *"in vitro"* relationship between the absorption of these two ions was found by Schachter and Rosen(2) who showed that concentrative calcium transport across the intestinal wall *in vitro* was depressed by the presence of magnesium in the medium. In conflict with this, Clark(3) reported that under normal dietary conditions, an increase of magnesium intake promoted calcium absorption rather than inhibiting it.

In the rat nutritional magnesium deficiency not only causes decreased concentrations of magnesium in serum and bone but also produces hypercalcemia, hypophosphatemia, hypocalciuria, hyperphosphaturia and renal calcification(4,5). To determine the relationship between intestinal function and the hypercalcemia and hypophosphatemia of the mag-

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