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Composition and Surface Activity of Normal and Phosphatidylglycerol-Deficient Lung Surfactant

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ABSTRACT. The possibility that pulmonary surfactant, characterized by a phosphatidylglycerol deficiency, as in early fetal life, might have inferior surface properties was evaluated. We obtained this specific surfactant from adult rabbits by withholding glucose and giving them an excess of myoinositol by mouth and intravenously. Controls were given a similar quantity of glucose. The myoinositol resulted in a drastic reduction of surfactant phosphatidylglycerol, from 7.2 to 0.3% of phospholipids, and a corresponding increase in phosphatidylinositol from 4.8 to 11.3%. In addition, the myoinositol treatment increased the myoinositol that was disaturated from 18.5 to 27.3% ($p < 0.05$). The corresponding figures for disaturated phosphatidylcholine were 56.0 and 60.5%, respectively (NS). The myoinositol treatment for 4 days increased the pool size of alveolar surfactant by 32% ($p < 0.01$). The surface activity was studied with modified Wilhelmy balance and the pulsating bubble surfactometer. Surfactant containing phosphatidylinositol rather than phosphatidylglycerol was not inferior, as compared to surfactant that contained phosphatidylglycerol (minimum surface tension: 2.0 *versus* 2.2 $mN \cdot m^{-1}$; collapse rate at 10 $nM \cdot m^{-1}$: 1.85 *versus* 1.95 min^{-1}; rate of adsorption from subphase to surface: 32 *versus* 35 $mN \cdot m^{-1} \cdot 30\ s^{-1}$), nor was there a difference in the ability of the two surfactants to improve lung stability of 27-day-old rabbit fetuses (air retention at 35 cm H_2O: 1.8 *versus* 1.8 ml/30 g; air retention at 0 cm H_2O: 0.8 *versus* 0.9 ml/30 g). We conclude that phosphatidylinositol surfactant does not have inferior surface properties. Myoinositol affects not only the acidic surfactant phospholipids but also increases the pool size of surfactant by an as yet unknown mechanism. (*Pediatr Res* 19: 286-292, 1985)

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Abbreviations

DPPC, dipalmitoyl phosphatidylcholine
PC, phosphatidylcholine
PG, phosphatidylglycerol
PE, phosphatidylethanolamine
PI, phosphatidylinositol

A main and very active component of pulmonary surfactant is DPPC. There are also other phospholipids present: monounsaturated PC, PG, PE, and PI. Free fatty acids, triglycerides, and cholesterol are also found in small quantities, and there is at least one specific protein (23). The composition of pulmonary surfactant appears rather similar among various mammalian species but can be affected by the serum concentration of myoinositol, a hexahydroxy derivative of cyclohexane. A high concentration of this sugar alcohol suppresses PG synthesis and correspondingly increases the PI content of surfactant in adult animals, *in vivo* as well as *in vitro* (3, 5, 16). In pregnancy, the fetal surfactant synthesis may be similarly affected, since the serum concentration of myoinositol may be up to 20 times higher than in the adult and permeability into alveolar cells may be increased (6, 31). These circumstances explain that in surfactant from immature fetuses PI is detectable, but PG is not. The effluent from airways of infants suffering from the respiratory distress syndrome is characterized by the presence of PI and the absence of PG, and it has been suggested that PG specifically improves the surfactant quality (18), but it is also possible that the appearance of this phospholipid in the lung effluent of a fetus near term merely indicates an overall maturity of the lung, including a larger size surfactant pool (13).

The purpose of this study was to clarify whether surface properties, as they affect lung expansion and stability, are inferior when the surfactant lacks PG but contains correspondingly more PI. Pulmonary surfactant from normal adult rabbits was compared with that synthesized in rabbits with a high serum myoinositol concentration.

MATERIALS AND METHODS

Obtaining rabbit surfactant. Male, New Zealand rabbits weighing 1.2 ± 0.1 kg, were used. Their food intake was *ad libitum*, but with the drinking water, and with two separate intravenous infusions, the animals also received either myoinositol or glucose.

Water was totally withheld for 14 to 16 h prior to the first infusion with a sterile solution of either glucose or myoinositol (Sigma, Inc., St. Louis, MO). The concentration of the solutions was 10% (w/v) or, since the molecular weight of both sugars is about 180, 0.56 M. The infusion, lasting approximately 3 h, was given into an ear vein in a volume of 40 ml/kg. The rabbits were then allowed to drink again, but only a 4% solution of the same kind of sugar that was used for the infusion, glucose or myoinositol. Only animals drinking more than 80 ml/kg/day were included in the study. Two to four days after the first infusion with glucose or myoinositol, they received a second identical infusion, before and after which free serum myoinositol was analyzed with gas-liquid chromatography (15).

Surfactant was recovered 14 to 16 h following completion of the second infusion. The animals received ketamine hydrochloride (45 mg/kg) and xylazine (15 mg/kg) and were bled through ear arteries. The trachea was cannulated under local anesthesia and the lungs lavaged five times with 0.9% NaCl (15–18 ml/kg for each lavage). The liquid obtained was centrifuged at 150 × *g* for 10 min. This and all subsequent steps took place at 0 to 4° C. Any lavage with visible blood contamination was discarded. The supernatant was centrifuged at 7500 × *g* for 120 min. The resulting pellet was resuspended in 0.9% NaCl and layered over a discontinuous density gradient containing 0.6 M sucrose, 20 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA (pH 7.4), and 0.27 M sucrose-NaCl-Tris-EDTA. After centrifugation at $10^5 \times g$ in an SW 27 rotor for 90 min, an interphase which developed between the two sucrose layers was collected, diluted with a large excess of distilled water, and subsequently centrifuged at 7500 × *g* for 120 min. The pellet was dispersed in water and a known volume of the suspension was freeze-dried so it could be shipped overseas.

Biochemical methods. The phospholipids and neutral lipids were analyzed as described previously (17–19). To improve the separation between PI and PC, two-dimensional thin-layer chromatography was used and the second direction was run twice. For further analysis of the phospholipids, the appropriate lipid spots were eluted from the silica gel after they had been visualized by spraying with water or methanol.

The phospholipid's fatty acid distribution was analyzed with gas-liquid chromatography, usng a Perkin-Elmer instrument with a column packed with 10% Silar 10C on 100/120 Mesh Gas ChromQ (Applied Science Laboratories, State College, PA). The carrier gas was N_2 and the isothermal temperature 180° C. The fatty acid methyl esters were identified using appropriate standards and mass spectrometry. The areas under the peaks were integrated with an M_2 Perkin-Elmer integrator. For analysis of the distribution of the fatty acids, the phospholipids were first treated with phospholipase A_2 (*Crotalus adamanteus*, Sigma), followed by analysis of the lysophospholipids and the free fatty acids. Saturated PC was quantified according to Mason *et al.* (17, 27).

The molecular classes of the phospholipids were further analyzed, essentially as described by Okano and Akino (35). Using phospholipase C from *Bacillus cereus* and *Clostridium welchii* (Sigma), the phospholipids were converted into 1,2-diacylglycerols (36), which were then extracted with ether from the incubation mixture. The dry extracts were immediately acetylated with acetic anhydride and anhydrous pyridine. The diacylglycerol acetates were recovered and purified as described by Kuksis *et al.* (26), and according to their degree of saturation they were resolved into molecular species by means of argentation thin-layer chromatography. Silica gel H plates containing 10% $AgNO_3$ were first developed to a height of 8 cm with chloroform/methanol (95:5 v/v), dried, and then developed to 20 cm with chloroform. The diacylglycerol acetates were recovered from the gel by the method of Arvidson (1). The fractions were analyzed for fatty acids by gas-liquid chromatography, using pentadecanoic acid as an internal standard. This allowed both qualitative identification of the molecular subclasses and quantitative analysis of the distribution of individual molecular subclasses.

Surface activity assessment. A Wilhelmy balance was used with a tight-fitting Teflon barrier, as described by Hildebran *et al.* (20). A temperature of 36.7 ± 0.3° C was maintained, and the subphase contained 5 mM Tris-HCl, 154 mM NaCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$ (pH 7.4). To avoid leaks with surface compression, the inside walls of the trough were treated with $LaCl_3$-DPPC (20). Dry surfactant containing 250 nmol phospholipid phosphorus was applied to the surface of 444 cm^2. From this maximal size, the surface area was cyclically compressed to a minimum of 40 cm^2 at a constant rate of $cm^2 \cdot s^{-1}$, and area/surface tension isotherms were obtained with an X/Y recorder. The collapse rate (K_m) of the film kept at minimum area was calculated according to Hildebran *et al.* (20): $K_m = 1/(\gamma_{eq} - \gamma) \times d\gamma/dt$; where γ_{eq} = equilibrium surface tension (26 $mN \cdot m^{-1}$); γ = 10 mN/m; $d\gamma/dt$ = rate of increase in surface tension at 10 mN/m. The lowest compressibility (C_m) at 10 mN/m was calculated as follows: $C_m = (1/A) \times (dA/d\gamma)$; where A = surface area at 10 mN/m.

The recruitment index was calculated according to Notter *et al.* (33): recruitment index = $[\gamma_{min} + (\Delta\gamma)]/\gamma_{min}$, where γ_{min} = minimum surface tension; $\Delta\gamma$ = surface tension change over the first 5% of the total expansion cycle.

The principle of the pulsating bubble surfactometer is to

continuously record pressure around a bubble, communicating with ambient air through a capillary and pulsating in the liquid to be examined (9). The bubble radius, r, cyclically varies in size from a maximum of 0.55 mm to a minimum of 0.4 mm. When the pressure difference across the bubble surface, ΔP, is known, as well as the corresponding value of r, which is measured with the greatest accuracy at maximal and minimal bubble size, then surface tension, γ, can be calculated from the law of Laplace: $\Delta P = 2\gamma/r$. The freeze-dried surfactant was suspended by vortexing in 100 mM NaCl and 2 mM $CaCl_2$ and the phospholipids were given a concentration of 10 or 2 μmol/ml. For evaluation with the bubble surfactometer, the 20 μl sample chamber was filled with this suspension and, while the recorder was running, a bubble was quickly expanded and made to pulsate at 20 rpm. The speed with which ΔP diminished after bubble creation reflected the rate of monolayer formation, *i.e.* adsorption rate. The temperature was 37° C.

The ability of the two types of surfactant to facilitate lung expansion and maintain stability was assessed by instilling the surfactant into the trachea of the preterm rabbit neonates. When the pregnancy had reached the age of 27 days and 16 to 20 h,

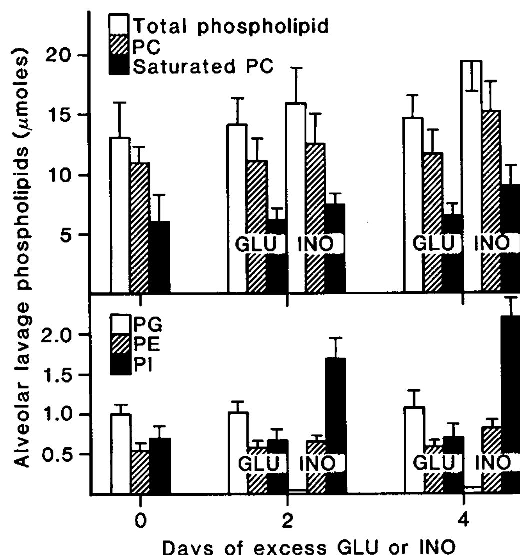

Fig. 1. The effect of the glucose (*GLU*) or the myoinositol (*INO*) treatment on the recovery of surfactant phospholipids in alveolar lavage. The results are given as mean ± SD of five to eight assays. Myoinositol for 4 days significantly increased surfactant phospholipids ($p < 0.02$), except PG.

the does were sacrificed with a fast iv injection of 10 ml thiopental sodium (20 mg/ml). The abdomen was opened immediately, and through the uterine wall the fetuses were injected intracranially with 10 mg thiopental sodium. This prevented them from gasping when delivered. The trachea was cannulated with a metal tube, length 12 mm, OD 1.25 mm. By holding up the hind legs of the rabbit pups, pulmonary fluid was made to flow from the metal tube via a fine polyethylene catheter directly into a sample chamber of the bubble surfactometer. Assessing surface properties of the pulmonary fluid from each neonate individually offered evidence that those tested with one surfactant were no different from those tested with the other, and both groups had immature lungs.

Lung expansion, as affected by the two types of surfactant, was studied simultaneously in all neonates of a litter, using the method of Enhorning and Robertson (10). A polyethylene catheter with 25 μl surfactant suspension, or 25 μl of saline, was connected to the metal tracheal tube, making sure that the pulmonary fluid in the latter and the surfactant were in direct contact with no air in between. The other end of the catheter communicated, via a horizontal glass tube containing 2 ml air, with a water reservoir. The latter was raised stepwise to a height of 10, 20, 30, and 35 cm and then lowered to 30, 20, 10, and 0 cm. Since each level of pressure thus created equally affected all neonates of a litter and was maintained for 15 s, it required 2 min to obtain the inflation-deflation curves for the whole litter. The newborn dead rabbits were all immersed just under the surface of water kept at 37° C. Air moving into lungs from any of the corresponding parallel glass tubes would be replaced with water from the reservoir. The increase in the amount of water in the horizontal glass tubes was recorded photographically and corresponded to the volume of air in a pair of lungs. For the final analysis, the air volume was related to 30 g body weight, the average for rabbits at a gestational age of 27 days.

Other methods. The results were expressed as mean ± SD. The statistical significance was calculated using one-way analysis of variance and the *t* test.

Table 2. *The percentage composition of the molecular subclasses of the PC*

	PG-surfactant ($n = 5$)	PI-surfactant ($n = 5$)
Saturated	56.0 ± 6.0	60.5 ± 7.2
Monounsaturated	30.5 ± 2.2	28.5 ± 3.3
Diunsaturated	8.5 ± 3.5	7.3 ± 1.2
Polyunsaturated	5.0 ± 2.9	4.0 ± 3.0

Table 1. *The percentage fatty acid composition of the neutral phospholipids in PG- and PI-surfactant*

	PG-surfactant ($n = 5$)		PI-surfactant ($n = 5$)	
	PC	PE	PC	PE
14:0	1.2 ± 0.4 (1.6, ND)*	2.0 ± 0.4	1.2 ± 0.5 (1.2, ND)	0.9 ± 0.5
14:1	0.5 ± 0.1 (0.7, ND)	1.0 ± 0.6	ND	1.3 ± 0.4
16:0	69.5 ± 3.6 (84.8, 53.1)	27.5 ± 3.5	72.1 ± 4.7 (87.2, 57.6)	28.0 ± 3.2
16:1	6.5 ± 0.8 (3.4, 9.6)	9.0 ± 1.0	6.0 ± 1.1 (3.3, 8.2)	8.2 ± 0.6
18:0	4.0 ± 0.3 (2.4, 5.2)	10.6 ± 0.7	4.5 ± 0.5 (3.6, 6.7)	12.4 ± 1.0
18:1	14.0 ± 1.9 (6.3, 24.5)	29.7 ± 2.9	12.8 ± 2.0 (4.1, 22.0)	30.0 ± 2.8
18:2	4.3 ± 1.0 (0.8, 7.5)	16.8 ± 2.9	3.4 ± 1.2 (0.6, 6.4)	15.7 ± 2.0
20:1	ND	1.8 ± 0.7	ND	1.6 ± 1.0
20:4	ND	0.8 ± 0.8	ND	1.0 ± 0.4
Unidentified	ND	0.8 ± 0.6	ND	0.9 ± 0.5
Saturated	74.7 ± 3.6 (88.8, 58.3)	40.1 ± 3.9	77.8 ± 4.7 (92.0, 64.3)	41.3 ± 4.0
Monounsaturated	21.0 ± 2.0 (10.4, 34.1)	39.7 ± 3.0	18.8 ± 2.9 (7.4, 30.2)	41.1 ± 3.6

* The figures in the parentheses indicate the percentage of the fatty acyl moieties associated with 1-, and 2-position, respectively. ND means less than 0.5% of the total fatty acids.

Table 3. *The percentage fatty acid composition of the acidic phospholipids in PG- and PI-surfactant*

	PG-surfactant (n = 5)			PI-surfactant (n = 5)
	PG	PI	PG + PI	PI
14:0	3.0 ± 0.7 (4.5, 1.8)	1.2 ± 0.4 (1.1, 1.3)	2.3 ± 0.5 (3.1, 1.6)	1.2 ± 0.1 (1.5, 2.0)
14:1	0.7 ± 0.5 (0.5, 0.5)	1.1 ± 0.1 (ND, 2.0)	0.9 ± 0.2 (ND, 1.1)	1.0 ± 0.2 (0.5, 1.5)
16:0	53.2 ± 2.4[1] (61.6, 47.3)	35.1 ± 1.9[1,2] (45.2, 26.2)	46.0 ± 2.0 (55.0, 38.9)	47.0 ± 2.7[2] (54.0, 39.0)
16:1	10.2 ± 0.9 (10.4, 10.1)	9.8 ± 0.7 (9.8, 9.2)	10.0 ± 0.8 (10.2, 9.7)	9.1 ± 0.4 (8.5, 10.6)
18:0	10.3 ± 1.3[1] (12.5, 8.5)	16.7 ± 2.0[1,2] (20.0, 13.1)	12.9 ± 2.1 (15.5, 10.3)	12.8 ± 1.1[2] (17.9, 6.9)
18:1	17.7 ± 1.9[1] (8.5, 24.1)	26.1 ± 2.5[1] (19.0, 33.9)	21.1 ± 2.5 (12.7, 28.0)	21.7 ± 1.9 (13.1, 28.8)
18:2	4.0 ± 1.0 (1.2, 6.8)	6.7 ± 0.3 (4.0, 9.0)	5.1 ± 0.6 (2.3, 7.7)	5.4 ± 0.8 (4.0, 7.4)
20:1	ND	0.9 ± 0.4 (ND, 1.3)	ND	ND
20:4	ND	ND	ND	ND
Unidentified	0.9 ± 0.4 (0.8, 0.9)	2.4 ± 0.9	1.5 ± 0.5 (0.8, 2.1)	1.8 ± 0.7 (0.5, 3.3)
Saturated	66.5 ± 2.9[1] (78.6, 57.6)	53.0 ± 4.0[1,2] (66.3, 40.6)	61.2 ± 3.2 (73.7, 50.8)	61.0 ± 3.4[2] (73.4, 48.9)
Monounsaturated	28.6 ± 1.6 (19.4, 34.7)	37.9 ± 3.2 (28.8, 46.4)	32.1 ± 2.9 (23.6, 39.4)	31.8 ± 2.3 (22.1, 40.9)

The identical superscripts on the same line indicate that the two groups differ significantly ($p < 0.02$) from each other. For other information see Table 1.

Table 4. *The percentage composition of the molecular classes of the acidic surfactant phospholipids*

	PG-surfactant (n = 4)			PI-surfactant (n = 4)
	PG	PI	PG + PI	PI
Saturated	31.5 ± 5.3[1]	18.5 ± 3.0[1,2]	26.3 ± 4.4	27.3 ± 3.9[2]
Monoenoic	52.8 ± 7.4	62.3 ± 6.7	56.6 ± 7.5	52.9 ± 4.2
Dienoic	13.3 ± 6.2	14.1 ± 2.9	13.6 ± 5.5	15.0 ± 3.8
Polyenoic	2.4 ± 1.0	5.1 ± 4.0	3.5 ± 1.4	4.8 ± 1.9

The identical superscripts on the same line indicate that the two groups differ significantly ($p < 0.05$) from each other.

RESULTS

Serum myoinositol shortly before and 12 to 14 h after the second intravenous infusion was 2.15 ± 0.40 and 3.67 ± 0.69 mM, respectively. The controls, receiving glucose, had a myoinositol concentration in serum of only 62 ± 9 μM.

Lipid composition. Figure 1 shows the phospholipid recovery in the total but cell-free alveolar lavage. Following myoinositol treatment for 4 days, all surfactant phospholipids, except PG, increased, whereas glucose infusion had no detectable effect on phospholipid pool size or distribution. The myoinositol treatment had a dramatic effect on the acidic phospholipids. Thus, PG decreased from 7.2 ± 0.9 to 0.3 ± 0.3% of total phospholipids, and PI increased from 4.8 ± 0.7 to 11.3 ± 2.9%.

Tables 1 and 2 show data on the fatty acid structure of the nonacidic phospholipids PC and PE. There is a higher percentage of saturated species in PC than in PE, but the fatty acid structure of these two nonacidic phospholpids was not affected by the myoinositol treatment.

Tables 3 and 4 show data on the fatty acid structure of the acidic phospholipids PG and PI. In the PG surfactant, *i.e.* the surfactant originating from glucose-infused rabbits, PG contained relatively more palmitic acid than did PI but less stearic and oleic acids. The PI surfactant, *i.e.* the surfactant originating from myoinositol-treated rabbits had more palmitic but less stearic and oleic acids. Myoinositol treatment significantly increased the saturated fatty acids of PI. The comparison of PG and PI surfactant shows that there was no detectable difference in the fatty acid structure of the total acidic phospholipids.

Surface properties. The *in vitro* surface properties as evaluated with the Wilhelmy balance and with the bubble surfactometer are reported with Table 5. PG and PI surfactants were very similar and fulfill all criteria reportedly typical to lung surfactant. Figure 2 illustrates typical area to surface tension isotherms of PG and PI. There is a small but significant difference which is also reflected in the recruitment index (Table 5).

With the pulsating bubble surfactometer, it was found that both types of surfactant had a fast adsorption rate. As soon as the bubble was expanded, a surface tension of 30 to 35 mN/m was recorded. During an "expiration" surface tension decreased and in the first cycle reached a value of 5 to 10 mN/m. After 1 min of pulsation, the lowest value of surface tension, coinciding with minimal bubble size, was close to zero (Fig. 3).

When the two types of surfactant were instilled into the upper airways of immature lungs, the pressure-volume loops obtained were almost identical (Fig. 4). There was little if any air expansion, hysteresis, or air retention at 0 cm H_2O when saline instead of surfactant was instilled to the airways.

DISCUSSION

With the present study, we have confirmed that by replacing glucose intake with myoinositol it is possible to alter the surfactant lipid composition of an adult animal toward that found in fetal life (16, 18). The surfactant originating from the rabbits receiving glucose by mouth and intravenously was similar to that of normal adult surfactant, containing PG as well as PI. On the other hand, rabbits receiving myoinositol by mouth and as an intravenous infusion had barely any PG in their surfactant, but did have correspondingly increased PI. The ratio between these acidic phospholipids was similar to what characterizes surfactant of early fetal life when the concentration of serum myoinositol is high. The myoinositol treatment resulted in an increased surfactant pool, but had no detectable effect on the relative concentration of other components than PG and PI.

In addition, the myoinositol treatment increased the saturated fatty acid moieties (especially palmitate) of PI, and the fatty acid structure of PI approached that of PG. Therefore, the myoinositol treatment did not significantly affect the fatty acid structure of the total acidic surfactant phospholipids (PG + PI), although there were severe perturbations in the individual acidic phospholipids. This is in accordance with the proposal that PG and PI derive the fatty acid moieties from a common CDP-diacylglycerol pool (16).

A previous study demonstrated that PI surfactant from fetal rabbit lung was surface active (18). However, it had a higher compressibility at low surface tensions than had PG surfactant from postnatal lung, suggesting a poor capacity to stabilize alveoli during expiration. Isolation of surfactant from lungs at different developmental stages may result in unpredictable nonsurfactant contamination, and several subtle differences in composition

Table 5. *Comparison of the surface properties of PG and PI-surfactants*

	PG-surfactant	PI-surfactant	$p<$
Wilhelmy balance			
Minimum surface tension (mN/m)	2.2 ± 0.2 (7)*	2.0 ± 0.8 (7)	
Lowest compressibility at 10 mN/m (m/mN)	0.035 ± 0.012	0.032 ± 0.001	
Surface concentration of phospholipid at 10 mN/m (nmol/cm^2)	1.04 ± 0.4	1.05 ± 0.06	
Recruitment index	13.2 ± 1.3	16.0 ± 1.9	0.01
Collapse rate at 10 mN/m (1/min)	1.95 ± 0.4	1.85 ± 0.2	
Decrease in surface tension during adsorption from subphase to surphase			
Δ mN/m after:			
30 s	12 (3),† 35 (2)‡	14 (3),† 32 (2)‡	
2 min	25, 37	20, 37	
5 min	32, 32	34, 36	
Pulsating bubble surfactometer			
Minimum surface tension			
mN/m after:			
1st pulse	14 (2),§ 6 (3)‖	15 (2),§ 7 (3)‖	
15 s	5, 0	2, 0	
30s	1, 0	0, 0	

* Mean ± SD (number of assays).
† The concentration of surfactant phospholipid 25 nmol/ml.
‡ The concentration of surfactant phospholipid 100 nmol/ml.
§ The concentration of surfactant phospholipid 2 μmol/ml.
‖ The concentration of surfactant phospholipid 10 μmol/ml.

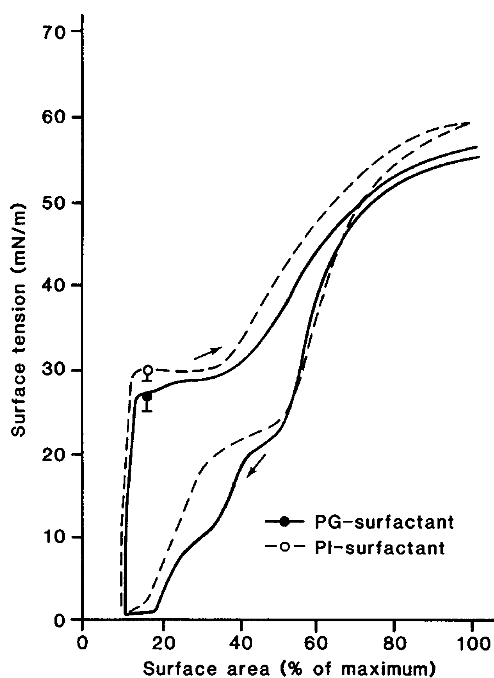

Fig. 2. Surface area–surface tension isotherms of PG and PI surfactants. The surface tension during expansion cycle (mean ± SD of seven measurements in each group) indicates a minor difference between PG and PI surfactants.

could have modified the surface properties. Therefore, there was still a possibility that PI surfactant had surface properties inferior to those of normal PG surfactant, and a comparison of the two types, both originating from adult lungs, seemed pertinent.

Our study demonstrated that both types of surfactant *in vitro* exhibited the following important properties (24): 1) a rapid adsorption from subphase to surface, 2) a minimal surface tension of 0 to 4 mN/m on compression of the surface, 3) a compressibility considerably less than the 0.09 mN/m at low surface tension (< 15 mN/m), 4) a slow collapse of the maximally compressed film toward the equilibrium surface tension, 5) a wide hysteresis loop observed with the Wilhelmy balance. Our observation that the surface properties of PG and PI surfactants were only marginally different was in accord with a previous report (4), and there was no clear evidence that one was superior to the other. Of several parameters analyzed, the only difference observed was a slightly higher recruitment index (33) of the PI surfactant. This minor difference is not necessarily due to the acidic phospholpids, and its effect was not noticeable on the compliance of premature rabbit lungs. The pressure-volume relationship was equally improved by the two types of surfactant (4). Our data are in accord with those reported by Beppu *et al.* (4) who also found a similarity in the ultrastructure of normal and PG-deficient surfactant. Both surfactant suspensions contained tubular myelin. It would seem, from our results and those of others, that PI can substitute for PG without affecting normal surfactant function.

The role of the acidic phospholipids is not clearly understood, but present evidence indicates that PG improves the function of saturated PC. The collapse of a DPPC film, occurring during compression, can be prevented by the presence of PG in the monolayer (2). This may explain why surfactant PG improves the stability of surfactant PC at low surface tension (22). PG may also affect the adsorption rate, in that the poor adsorption of a liposome suspension prepared from DPPC only, is effectively improved by the inclusion of PG (34). These effects of PG, detected with physical methods, may explain why PG to some extent improves the surface properties of DPPC during a surfactant substitution (21, 30). However, it is evident that also other lipids, especially PI, improve the function of the major surfactant phospholipid (21, 22, 28, 32, 37), and so far there is no convincing evidence to indicate tht PG is superior to PI as a component of an artificial surfactant (12, 22, 25, 28, 34).

Our results do not exclude the possibility that PG has an

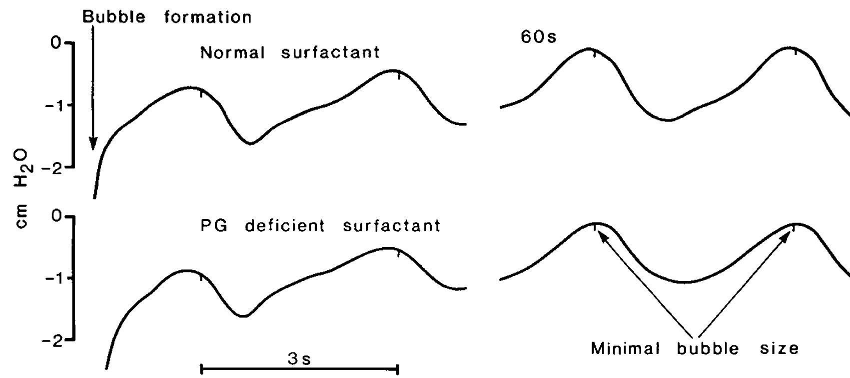

Fig. 3. Pressure tracing obtained with the pulsating bubble surfactometer when the bubble was pulsating in normal and PG-deficient surfactant (2 μmol phospholipid/ml). Fast adsorption was noted with both types and, 1 min after bubble formation, surface tension was close to zero at minimal bubble size.

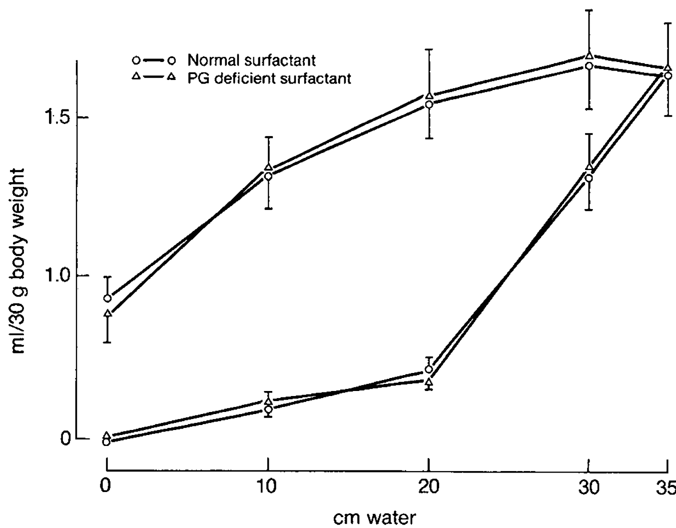

Fig. 4. Pressure volume loops from preterm rabbit neonates given a tracheal instillation of normal (n = 24) and PG-deficient (n = 25) surfactant. Lung compliance was equally affected by the two types of surfactant. Instillation of saline without surfactant into the airways allowed a maximum of 0.4 ± 0.3 ml/30 g of air entry and less than 0.1 ml/30 g of air retention at 0 cm H_2O.

important role in lipid metabolism; in fact, there would seem to be a difference in the ability of PI and PG to affect the synthesis (11) as well as the intracellular transport and secretion (17) of surfactant.

A remarkable finding of the present study was that myoinositol treatment for 4 days not only alters the acidic phospholipids but also significantly increases the alveolar surfactant pool. Interestingly, myoinositol also increases the synthesis and secretion of surfactant PC during hormone-induced fetal lung maturation (15). This consequence of the high fetal serum (extracellular) myoinositol would seem to be beneficial. However, in healthy individuals there are no known benefits attributable to an increase of the surfactant pool. Furthermore, excess myoinositol may have damaging effects on other organ systems. *In vitro* myoinositol has been shown to promote the growth of malignant as well as normal cells (8), and it has been proposed that myoinositol excess may cause neuropathy (7, 14). If myoinositol is ever considered for therapy to promote surfactant synthesis, these potential side effects must be taken into consideration.

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Postheparin Plasma Lipases and Carnitine in Infants during Parenteral Nutrition

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ABSTRACT. Lipoprotein lipase is the rate-limiting factor for hydrolyzing triglycerides to glyerol and fatty acids. Carnitine is a cofactor in the transport of long-chain fatty acids through the mitochondrial membrane for oxidation. To assess these determinants of fat utilization during total parenteral nutrition, lipoprotein and hepatic lipase activities and carnitine concentrations of nine newborn infants, operated on because of gastrointestinal anomalies during the first day of life, were measured with specific methods. Total parenteral nutrition was built up in 3 days whereafter the infants received 3 g/kg of fat at a constant rate of infusion for 24 h/day. Lipoprotein lipase activity of postheparin plasma increased from 14 to 35 μmol free fatty acids/ml/h during parenteral nutrition whereas hepatic lipase activity remained unchanged at 40 μmol free fatty acids/ml/h. Serum free carnitine and acylcarnitine levels decreased significantly during parenteral nutrition; urinary excretion of carnitine decreased also. In addition, serum cholesterol and phospholipids increased markedly during parenteral nutrition whereas serum triglycerides, free fatty acids, and blood β-hydroxybutyrate remained unchanged. Serum apolipoprotein A-I concentrations were unaltered, apolipoprotein A-II underwent a transient increase, and apolipoprotein B increased monotonically during parenteral nutrition. The results suggest that under the present circumstances neither lipoprotein lipase activity nor carnitine resources are rate-limiting for the utilization of fat in newborn infants during total parenteral nutrition. (*Pediatr Res* 19: 292–296, 1985)

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Lipoprotein lipase is the rate-limiting factor for clearance of fat from the circulation; it hydrolyzes triglycerides of lipoproteins and fat emulsion particles to glycerol and free fatty acids (4, 19). Carnitine is essential for facilitated transport of long-chain free fatty acids across the mitochondrial membrane (9).

Lipoprotein lipase in newborn infants is usually estimated by determining postheparin plasma lipolytic activity (6, 8). Postheparin plasma, however, contains hepatic lipase too (14, 26). The function of hepatic lipase is unsettled (11, 15, 16, 18, 19) but its activity in infants is about three times the activity of lipoprotein lipase (26, 27). In addition, these two lipases can vary independently (26, 27). Hence, postheparin plasma lipolytic activity is an inadequate measure of lipoprotein lipase.

Adults can synthesize the carnitine they need. Therefore, carnitine is not considered an essential nutrient. Newborn infants may not, however, be capable of sufficient carnitine synthesis. Therefore, infants may be dependent on nutritional sources of carnitine, such as milk. Infants receiving carnitine-free total parenteral nutrition are at risk of developing carnitine deficiency characterized by decreased blood concentration and tissue content of carnitine (2, 20, 24, 28). Reduced carnitine intake may impair fatty acid oxidation and diminish ketogenesis after fat infusion; these deficiencies can be corrected with supplementary carnitine (23, 29).

To study the role of the principal regulators of fat metabolism in newborn infants lipoprotein lipase and hepatic lipase activities and carnitine concentrations were measured during parenteral nutrition.

PATIENTS AND METHODS

Patients. The patients were nine newborn infants who required surgical operation and parenteral nutrition because of gastrointestinal tract anomalies (Table 1). Surgery was performed under general anesthesia during the first day of life. For subsequent parenteral nutrition seven infants received central venous catheters and two infants (patients 4 and 5) peripheral venous catheters; none had a continuous heparin infusion. After the opera-