Effects of hexoprenaline on the lecithin/sphingomyelin ratio and pressure-volume relationships in fetal rabbits

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A placebo-controlled, double-blind trial was carried out on 74 New Zealand White rabbit fetuses from 15 does to assess the effect of a fetal injection of hexoprenaline on surfactant release. After the uterus was exposed, half the fetuses received 0.1 ml (0.25 μg) of hexoprenaline injected intraperitoneally through the intact uterine wall; the other half received an equivalent volume of placebo. After 3 hours, the abdomen was reopened, and the fetuses were surgically delivered and killed before breathing. The lecithin/sphingomyelin (L/S) ratios, obtained from lung washings, revealed a mean of 1.59:1 for the placebo group and 1.92:1 for the hexoprenaline group ($p < 0.001$). Pressure/volume curves were generated from the lungs of 24 fetuses from 10 does, and the volume of air in the lungs for each pressure was analyzed in four ways: total volume, volume per gram of fetal body weight, volume per gram of dry lung weight, and as a percentage of total lung capacity at a pressure of 40 cm H_2O. A first and second inflation-deflation curve was obtained for each experiment. The lungs from the hexoprenaline-treated group retained significantly more air than those from the placebo group. The most significant comparison was obtained when lung volume was expressed per gram of dry lung weight. The possibility of administering a β_2-sympathomimetic drug to the mother in advanced preterm labor, specifically to release surfactant in the fetal lung, is suggested. (AM. J. OBSTET. GYNECOL. 139:726, 1981.)

SINCE THE PIONEERING STUDY of Liggins and Howie,[1] it has been confirmed[2-4] that the maternal administration of glucocorticoids, at least 24 hours before delivery, will favorably influence fetal lung maturity. However, this form of therapy has two major disadvantages: (1) Animal studies have indicated that corticosteroids cause temporary inhibition of lung growth[5, 6] as well as a reduced number of brain cells and disturbances of myelination, synaptic growth, and locomotor ability[7]; (2) corticosteroids require at least 24 hours to exert their effect,[8] thus obviating its use in the patient in advanced preterm labor.

Although enough surfactant is thought to be present in the granular pneumocytes of the lung by 30 weeks of gestation to line the air spaces up to 40 times,[9] it has to be secreted from the storage sites to protect against hyaline membrane disease. Drugs which increase adenosine 3′,5′-monophosphate (cyclic AMP) levels may be beneficial as it has been suggested that cyclic AMP is an important compound in the synthesis and release of surfactant.[10, 11]

The β-sympathomimetic drugs enhance the level of

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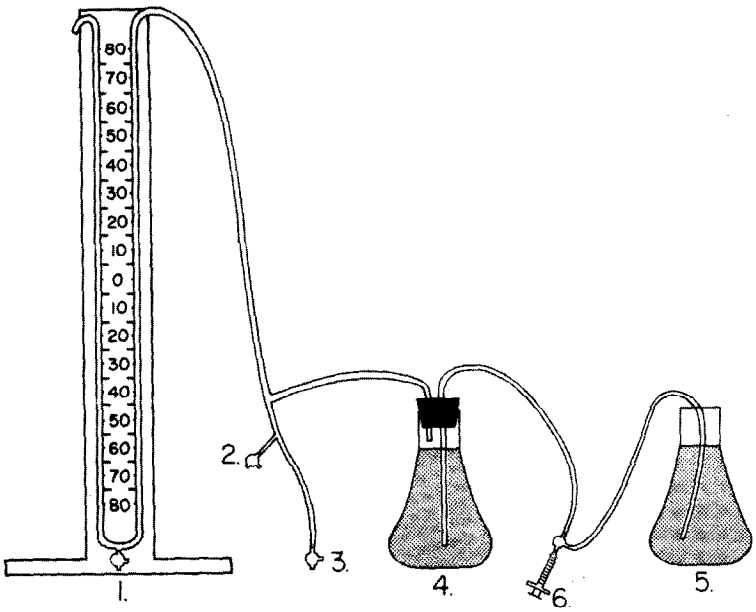

Fig. 1. Apparatus for pressure-volume studies. *1*, Water manometer; *2*, stopcock for equilibration; *3*, connection to fetus; *4*, closed water reservoir; *5*, open water reservoir; *6*, 1 ml water-filled syringe for calculation of displaced air volumes.

cyclic AMP by the stimulation of adenyl cyclase. The advantage of these drugs, compared to the steroids, is that release of surfactant may occur within a few hours after administration. Isoxsuprine was shown to enhance fetal lung maturity in the rabbit, whether administered chronically to the doe[12] or 3 hours after fetal injection.[13] In retrospective human studies, it has been shown that infants of mothers treated with either isoxsuprine,[14] salbutamol,[15] ritodrine,[16] or terbutaline,[17] to inhibit preterm labor, have had a reduced incidence of hyaline membrane disease.

Hexoprenaline, a β_2-sympathomimetic drug having fewer maternal cardiovascular effects than either fenoterol, ritodrine, or salbutamol,[18, 19] was used in a placebo-controlled, double-blind study to assess its effects on the biochemical and biophysical parameters of the rabbit fetal lung.

Methodology

Experiments were conducted on 74 New Zealand White rabbit fetuses from 15 does at a gestational age of 28 days, term being 31 days. Each doe was anesthetized by an intramuscular injection of ketamine (Bristol) and xylazine (Haver-Lockhart) in combination at 35 and 5 mg/kg of body weight, respectively.

The uterus was exposed via a midline abdominal incision under clean operating conditions. Beginning alternately on the left or right side, half the fetuses were consecutively injected intraperitoneally through the intact uterine wall with 0.1 ml (0.25 μg) of hexoprenaline sulfate. The other fetuses served as placebo controls and were injected with the same volume of equivalent vehicle without the hexoprenaline. The vials of active drug and placebo could not be distinguished by the investigator. For pH adjustment, 0.2 ml of 0.1M sodium phosphate buffer was added to 10.0 ml of hexoprenaline sulfate. The abdominal incision was closed with 2-0 silk and the doe was placed in a holding cage.

After 3 hours, the abdomen was reopened with the animal under the previously described anesthesia. The fetal rabbits with placentas and intact amniotic sacs were delivered surgically. After viability was established, and while still in the amniotic sac, the fetuses were immediately killed by an intraperitoneal injection of 0.1 ml of sodium pentobarbital (50 mg/ml), without breathing air. Nonviable fetuses were discarded. All fetuses were blotted dry and weighed. The doe was killed after delivery by an intracardiac injection of 1.0 ml of sodium pentobarbital, followed by 5.0 ml of a saturated potassium chloride solution.

A tracheostomy was performed on each fetus with the use of a short length of plastic tubing (Tygon S-54-HL; outside diameter, 054; inside diameter, 034).

Table I. Comparison of the L/S ratios and fetal weights in the hexoprenaline and placebo groups in 37 fetal pairs from 15 does

	Hexoprenaline		*Placebo*	
Litter No.	*Fetal weight (gm)*	*L/S ratio*	*Fetal weight (gm)*	*L/S ratio*
1	43.263	1.3	33.276	1.3
2	36.539	1.7	34.108	1.3
3	33.780	1.5	34.265	1.3
	31.554	1.5	29.918	1.3
4	26.300	1.3	29.008	1.3
	28.224	1.5	33.859	1.3
5	33.040	1.7	32.087	1.5
6	37.254	2.0	37.140	2.5
	33.541	1.7	34.948	1.5
7	34.440	2.0	35.100	1.5
8	30.701	1.7	30.850	1.5
	33.325	1.7	32.175	1.7
9	36.046	2.0	38.148	1.7
	34.285	1.7	37.648	1.5
	31.842	1.7	35.098	1.5
10	34.661	2.3	37.569	1.7
	30.434	2.5	35.720	1.7
	28.117	1.7	34.556	2.0
	27.717	2.0	28.359	2.0
	21.663	2.5	22.338	1.5
11	39.063	2.3	41.462	1.7
	36.779	1.7	36.626	2.0
	37.228	2.0	39.151	1.7
	34.475	2.0	34.454	1.5
12	41.039	2.0	42.877	1.5
	37.940	2.0	40.252	1.7
	36.345	2.5	37.187	1.5
13	35.969	2.0	34.455	1.7
	31.651	2.0	31.502	1.7
	31.487	1.7	30.117	1.5
	27.972	2.3	27.244	1.5
14	33.292	2.3	36.731	1.5
	33.100	2.5	34.969	1.7
	27.939	2.0	33.840	1.5
15	37.570	2.0	37.280	1.5
	38.609	1.8	39.292	1.5
	41.164	2.0	45.253	1.5
Mean	33.739	1.921	34.834	1.598
S D	4.564	0.325	4.700	0.241
P value*	0.028	0.001		

*By paired t test.

Pressure-volume inflation and deflation curves were obtained from randomly selected fetuses in both groups and paired according to weight. If possible, the contiguous, in-utero fetuses on the interface of the two groups were discarded to prevent any possible "carry-over" effect of the hexoprenaline.

In order to obtain the air pressure-volume curves, the tracheostomy tube was attached to a narrow-bore water manometer through one end of a T-tube and a three-way stopcock. The other end of the T-tube was connected to a water (H_2O) reservoir. Since the volume of air in the reservoir, tubing, manometer, and lung, when inflated, is constant, with only the distribution between the reservoir and lung varying, a compression factor could be determined for the system. Compression factors were determined by injecting H_2O into the reservoir and recording the volumes required to raise the air pressure in the system to 40 cm H_2O in steps of 5 cm H_2O, with the air passage to the fetus closed. A plastic, calibrated 1.0 ml syringe was used to inject H_2O into the closed system. Balancing the manometer at zero was facilitated by a three-way stopcock which could be opened to the atmosphere (Fig. 1).

Table II. Comparison of the fetal and dry lung weights of the hexoprenaline and placebo groups in the 12 fetal pairs in which air pressure-volume studies were performed

	Hexoprenaline		*Placebo*	
Litter No.	*Fetal weight (gm)*	*Dry lung weight (gm)*	*Fetal weight (gm)*	*Dry lung weight (gm)*
3	33.780	0.130	34.265	0.120
	27.544	0.110	34.347	0.140
6	37.254	0.100	37.140	0.120
	33.541	0.090	34.948	0.120
7	34.440	0.117	35.100	0.142
8	33.325	0.107	30.850	0.102
10	34.661	0.122	34.556	0.130
11	36.779	0.116	36.626	0.117
12	41.039	0.110	42.877	0.137
13	31.487	0.097	31.502	0.074
14	33.100	0.090	34.969	0.119
15	37.570	0.127	37.280	0.125
Mean	34.543	0.110	35.372	0.121
S D	3.40	0.01	3.06	0.02
P value	0.221	0.066		

After the system was balanced at zero pressure the air passage to the fetus was opened and the lungs were inflated to a maximal pressure of 40 cm H_2O, in steps of 5 cm H_2O. Syringe readings at each pressure were recorded after the lungs were allowed to equilibrate. Equilibration was defined as a stabilization of the column of water at each pressure for a period of 1 minute. An attempt to maintain this stabilization at each pressure was carried out for a maximum of 10 minutes at each pressure from 5 to 30 cm H_2O. At pressures of 35 and 40 cm H_2O, the maximum time allowed ranged from 1 to 5 minutes. By subtracting the compression factors from the volume of water injected into the system at each pressure, the volume of air displaced into the lungs could be determined for each pressure. The volume of air in the lungs at 40 cm H_2O was defined as total lung capacity (TLC). A deflation curve was obtained by returning to zero pressure in

Table III. Mean ± standard deviation of lung volumes of air at an inflation pressure of 40 cm H_2O

Measurement	*First inflation*		*Second inflation*	
	Hexoprenaline	*Placebo*	*Hexoprenaline*	*Placebo*
ml/air	2.65 ± 0.40	2.50 ± 0.54	3.46 ± 0.41	3.40 ± 0.61
	P = 0.385		P = 0.732	
ml/gm fetal body weight	0.077 ± 0.012	0.071 ± 0.014	0.101 ± 0.0131	0.096 ± 0.014
	P = 0.195		P = 0.279	
ml/gm dry lung weight	24.50 ± 4.73	21.18 ± 5.11	31.87 ± 4.53	28.59 ± 4.92
	P = 0.009		P = 0.008	

Table IV. Comparison of P values in hexoprenaline and placebo groups

Pressure (cm H_2O)	*Volume of air (ml)*		*Volume air (ml)/dry lung weight (gm)*		*Volume air (ml)/fetal body weight (gm)*		*Percentage of TLC*	
	Curve		*Curve*		*Curve*		*Curve*	
	First	*Second*	*First*	*Second*	*First*	*Second*	*First*	*Second*
0	1.000	0.043*	1.000	0.012*	1.000	0.009*	1.000	0.053
5	0.200	0.001*	0.302	0.001*	0.212	0.001*	0.208	0.001*
10	0.061	0.001*	0.105	0.001*	0.065	0.001*	0.055	0.001*
15	0.107	0.016*	0.264	0.001*	0.106	0.009*	0.078	0.005*
20	0.380	0.005*	0.609	0.001*	0.406	0.003*	0.267	0.001*
25	0.872	0.054	0.314	0.001*	0.742	0.024*	0.992	0.007*
30	0.471	0.226	0.130	0.033*	0.354	0.090	0.562	0.195
35	0.295	0.286	0.023*	0.001*	0.182	0.074	0.377	0.016*
40	0.385	0.732	0.009*	0.008*	0.195	0.279		
35	0.404	0.755	0.014*	0.009*	0.216	0.299	0.684	0.945
30	0.406	0.738	0.016*	0.009*	0.221	0.289	0.587	0.754
25	0.409	0.855	0.018*	0.020*	0.222	0.389	0.557	0.679
20	0.322	0.682	0.014*	0.016*	0.169	0.281	0.158	0.409
15	0.227	0.531	0.014*	0.027*	0.119	0.199	0.082	0.252
10	0.129	0.280	0.014*	0.015*	0.068	0.083	0.050*	0.081
5	0.059	0.062	0.014*	0.012*	0.038*	0.018*	0.035*	0.043*
0	0.043	0.950	0.012*	0.547	0.009*	0.899	0.216	0.922

*In all cases of a P value of 0.05 or less, the volume of air was greater in the hexoprenaline-treated group than in the placebo-treated group.

increments of 5 cm H_2O. Time for equilibration of the lungs during deflation was very short at high pressures, but at lower pressures it could be longer, up to 10 minutes. After the lungs were equilibrated at zero pressure, the air passage to the fetus was closed. The level of water in the manometer reservoir was readjusted to the starting mark; the manometer was balanced at zero, and a second inflation-deflation curve was determined.

Rupture of the lung was indicated by a rapidly falling column of water at higher pressures. This was confirmed by opening the abdominal cavity to inspect the diaphragm. In cases where the lungs had burst, the thin diaphragm was distended and bulging into the abdominal cavity because of air in the thoracic cavity. These fetuses were discarded.

On completion of the pressure-volume curves, lung washings were performed on most of the fetuses, including those for which no curves were obtained. The washings were repeated five times, with the use of 1.0 to 2.0 ml of physiologic saline, based on 1.0 ml/20 gm of body weight. The fluid from all five washings was combined and frozen until analyzed to determine the lecithin/sphingomyelin (L/S) ratio. The method used has been described elsewhere.[20]

After all lung washings, the thorax was opened and the lungs were extracted, blotted dry, and weighed (Mettler, PN 323). The lungs were then oven dried at 80° C for 48 hours, and the dry weight was recorded.

The volumes of air at each pressure were recorded as milliliters of air, milliliters of air per gram of fetal body weight, milliliters of air per gram of dry lung weight, and as the percentage of TLC.

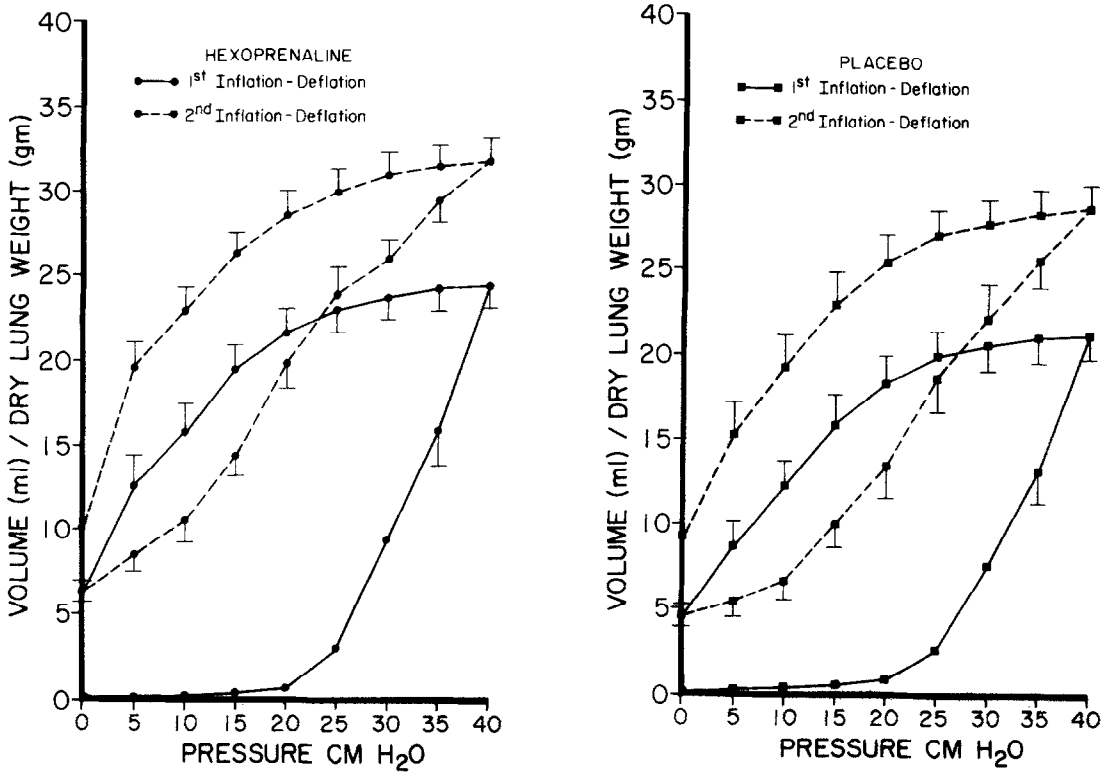

Fig. 2. Comparison of inflation-deflation curves between hexoprenaline and placebo groups. Standard errors are included where hexoprenaline and placebo groups are significantly different ($P < 0.05$).

A paired t test was used to test for significance between the drug and placebo.

Results

L/S ratios. The L/S ratios were determined on lung washings of 74 fetuses from 15 does. One half of the fetuses from each doe received an intraperitoneal injection of hexoprenaline and the other one half received a placebo. Table I lists the fetal weights and L/S ratios of each fetus. Sibling pairings for the paired t test were made as indicated in Table I by being listed on the same line. Where there was more than one pair from the same doe, pairings were made to match weights as close as possible. The average body weight of the placebo-treated fetuses was approximately 1.1 gm greater than that of the hexoprenaline-treated group. This difference was significant ($P = 0.028$) when analyzed by the paired t test. The mean value for the L/S ratios of the hexoprenaline-treated fetuses was 1.92:1 compared to a mean of 1.59:1 for the placebo-treated groups. This difference was highly significant ($P < 0.001$). An L/S ratio of 2.0:1 or greater was achieved in 21 hexoprenaline-treated fetuses compared to four from the placebo group ($\chi^2 = 15.46$, $P < 0.001$). There was no significant correlation between body weight and L/S ratios within either the hexoprenaline-treated group ($P = 0.74$) or the placebo-treated group ($P = 0.35$).

Pressure/volume relationships. Pressure/volume curves were generated from the lungs of 24 fetuses from 10 does. One or two pairs were selected from each litter after the fetuses had been weighed. Pairings were made to match the weight of hexoprenaline- and placebo-treated siblings as closely as possible. These pairings are indicated in Table II by being listed on the same line. Table II lists the fetal weights and dry lung weights of each fetus. There was no significant difference between the average body weights or the dry lung weights of the hexoprenaline and placebo groups.

The pressure/volume curves generated from the two groups were statistically analyzed by comparing the volume of air in the lungs for each pressure. Volumes were compared as total volume, volume per gram of fetal body weight, volume per gram of dry lung weight, and as a percentage of total lung capacity at a pressure of 40 cm H_2O. Table III lists the mean lung volumes of air at a pressure of 40 cm H_2O, with the volumes expressed in the three different ways. Table IV gives the

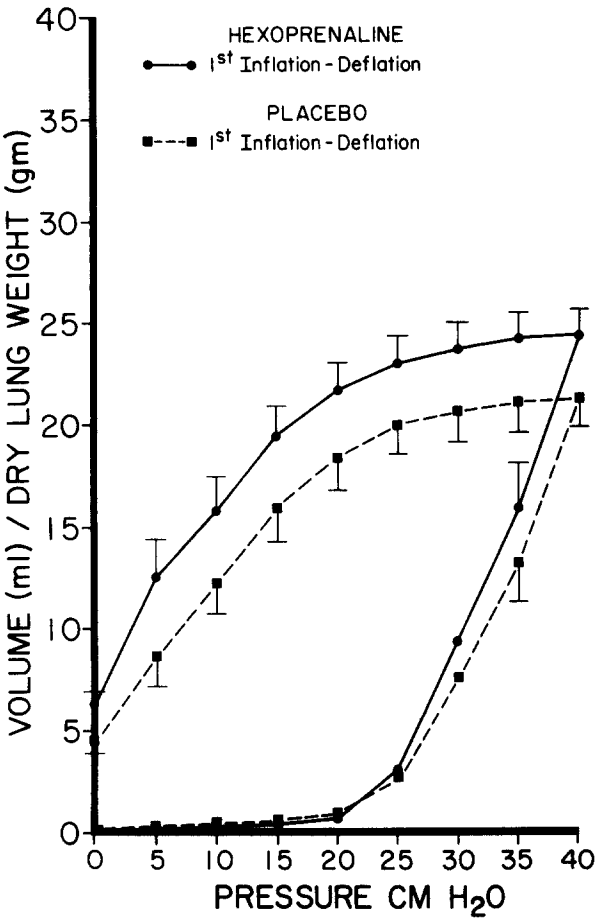

Fig. 3. Comparison of first inflation-deflation curves between hexoprenaline and placebo groups. Standard errors are included at points of significant difference between the two groups.

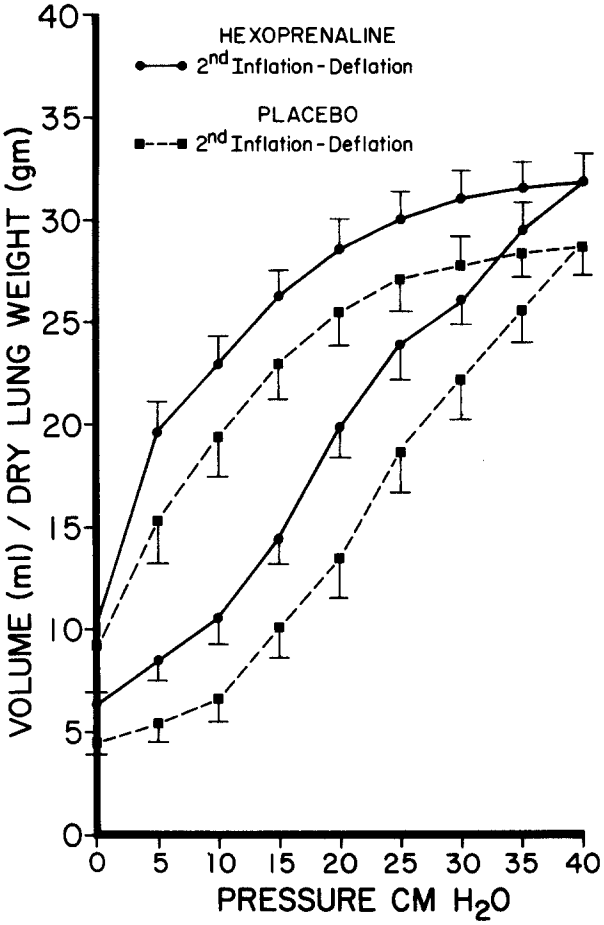

Fig. 4. Comparison of second inflation-deflation curves between hexoprenaline and placebo groups. Hexoprenaline group retained significantly more air at all pressures except at zero pressure of deflation curve.

P value of each pressure for the four different ways of comparing the lung volumes of the hexoprenaline- and placebo-treated groups.

Fig. 2 illustrates the first and second pressure/volume curves for both groups with lung volume expressed as milliliters per gram of dry lung weight. In both cases, the lungs accepted during inflation and maintained during deflation a greater volume of air for a given pressure during the second inflation than during the first inflation. Fig. 3 compares the pressure/volume relationship of hexoprenaline- and placebo-treated groups during the first inflation and deflation with the volumes expressed per gram of dry lung weight. During inflation the volumes were not significantly different until inflation pressures of 35 and 40 cm H_2O were reached. During deflation, the lung volumes were significantly different at all pressures.

Fig. 4 compares the pressure/volume relationship of the two groups during the second inflation and deflation. At all pressures, except zero pressure of the deflation curve, the volume of air in the hexoprenaline-treated group was significantly greater than the placebo-treated group.

Fig. 5 compares the pressure/volume relationship of the hexoprenaline- and placebo-treated groups during the first inflation and deflation with the volume expressed as a percentage of TLC at a pressure of 40 cm H_2O. The only significant differences were at pressures of 5 and 10 cm H_2O during deflation.

Fig. 6 compares the pressure/volume relationship of the two groups during the second inflation and deflation with the volumes expressed as a percentage of TLC at a pressure of 40 cm H_2O. Most of the points were significantly different during inflation, but during deflation the only significant difference was at a pressure of 5 cm H_2O.

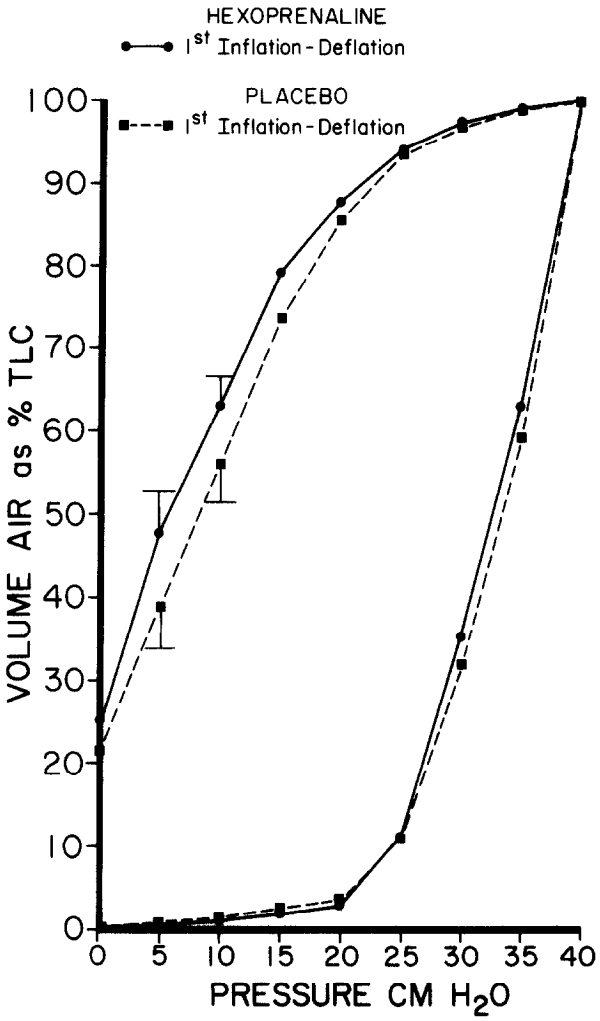

Fig. 5. Comparison of first inflation-deflation curves with the volume of air expressed as the percentage of TLC. Significant differences occurred only at 5 and 10 cm H_2O during deflation.

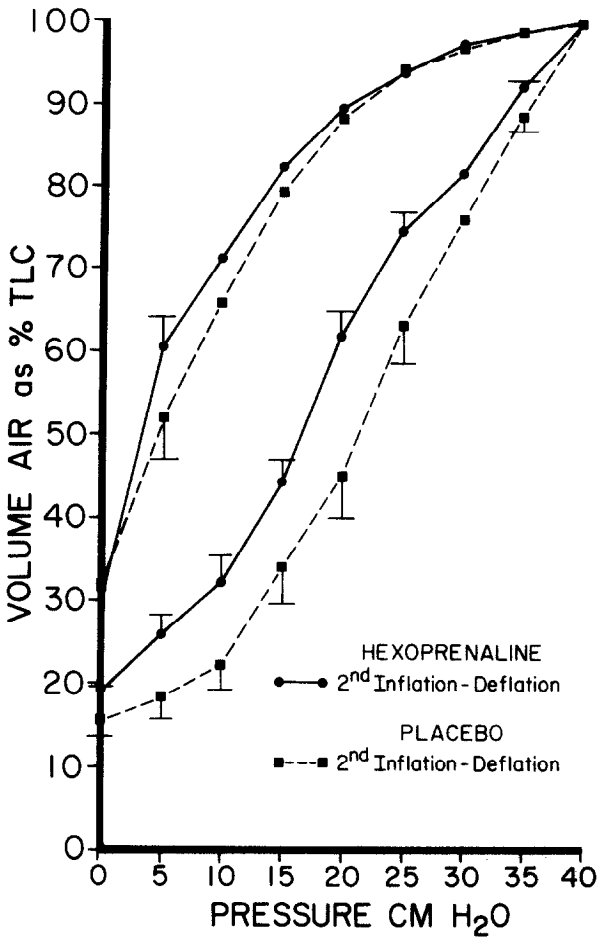

Fig. 6. Comparison of second inflation-deflation curves with the volume air expressed as the percentage of TLC. Most points were significantly different during inflation, but during deflation they were different only at 5 cm H_2O.

Comment

The L/S ratio of lung washings and the pressure/volume relationship of the lungs were used as an indirect measure of the amount of surfactant present in the lungs. Of course, the greater the L/S ratio, the greater the amount of surfactant present. The pressure/volume relationship of the lung when inflated with air is useful as an indirect measure of surfactant because of the surface tension–reducing property of surfactant. Within certain physiologic ranges, there is a direct relationship between the quantity of surfactant and the volume of air either accepted or retained in the lungs at a given pressure. Because of the hysteresis phenomenon produced by surfactant, the inflation and deflation limbs of the pressure/volume curve must be compared independently. In this experiment, both the chemical data (i.e., L/S ratio) and the physical data (i.e., pressure/volume curves) clearly demonstrate that more surfactant was present in the lungs of the hexoprenaline-treated group than in the placebo-treated group. It is important to note that the fetuses in this experiment were at an age (28 days' gestation) when surfactant production in the lung had already begun. Both the L/S ratio and the hysteresis present on the pressure/volume curves of the placebo group demonstrate the presence of surfactant. The fact that the hexoprenaline-treated group had more surfactant than the placebo-treated group only 3 hours after the administration of hexoprenaline strongly suggests that the increase is due to the release of surfactant already present in the

type II pneumocytes and is not likely due to an increased de novo production of surfactant.

Because of the varied ways the pressure/volume relationships of the lungs are expressed in publications reporting similar types of studies, it was decided to express the data obtained in these studies four different ways in order that comparisons could be made with other reported studies and to demonstrate the possible significance of selecting the best method. Table IV lists the P values obtained from a paired t test when the placebo- and hexoprenaline-treated groups were compared by the four methods. The most significant comparison was obtained when lung volume was expressed per gram of dry lung weight. This method of comparison eliminates the variability in animal weights and the biological variability between body size and lung size which can overshadow the change produced by the experimental variable. Even with the less precise methods of comparison significantly more air was retained by the lungs of the hexoprenaline-treated group at the end of the first deflation. However, when the lung volume is expressed per gram of dry lung weight, it can be demonstrated that there is significantly more air in the lungs of the hexoprenaline-treated group toward the end of the first inflation and throughout the entire deflation portion of the first curve, thus more clearly demonstrating the lower surface tension in the hexoprenaline-treated group.

During the first inflation of the fluid-filled fetal lung, surface tension forces and, therefore, the surface tension–reducing property of surfactant are not factors until air enters the alveoli and the liquid-to-air interface necessary for surface tension is present. As was the case in these studies, the quantity of surfactant present could not significantly affect the inflation limb of the first pressure-volume curve, except possibly at high pressures and volumes, therefore, it alone is not a reliable indicator of the amount of surfactant present. However, during the second inflation, when a liquid air interface is present, there is a direct relationship between lung volume at a given pressure and the quantity of surfactant present. In these experiments, during both the second inflation and the second deflation, there was less surface tension in the lungs of the hexoprenaline-treated group than in the placebo-treated group.

When lung volume is expressed as a percentage of total lung volume at the highest pressure, the difference in lung volume at the highest pressure is eliminated and only the shapes of the deflation curves are compared. In such a comparison, a difference in lung surface tension forces could only be demonstrated at low pressures since the differences at high pressures have been mathematically eliminated or reduced. In these experiments, the difference was significant at a pressure of 5 and 10 cm H_2O on the first deflation and 5 cm H_2O on the second deflation. For a similar reason, the significant difference consistently present in lung volumes between the two groups at the lower pressures of the second inflation is due to the fact that there was a significantly greater volume of air remaining in the lungs of the hexoprenaline-treated group at the end of the first deflation.

The fact that the fetal weights of the placebo-treated group listed in Table I are significantly greater than those of the hexoprenaline-treated group is coincidental and not likely related to any experimental procedure. Since selection as to which animals would receive the hexoprenaline or the placebo was made randomly through the intact uterine wall, it was not possible to select the fetuses according to size. It could be considered fortunate, however, that the placebo group was larger since lung maturity could possibly be equated with fetal size, in which case the group with the larger animals might be expected to have more surfactant present in the lungs. Of course, the reverse was found in these experiments.

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