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The Effects of Bumetanide and Furosemide on Lung Liquid Secretion in Fetal Sheep[1] (42276)

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Abstract. Thirty-four experiments were carried out on the effects of loop diuretics on lung liquid secretion in 20 fetal sheep (128–145 days gestation) with indwelling catheters. Bumetanide placed in the lung liquid at $2.19 \pm 0.52 \times 10^{-4}$ *M* produced immediate reabsorption of fluid, and effects lasted 3 hr ($n = 6$). Bumetanide at $1.1 \pm 0.17 \times 10^{-5}$ *M* reduced secretion significantly for 2 hr ($n = 4$), but at $1.07 \pm 0.06 \times 10^{-6}$ *M* there was no clear effect ($n = 6$). Controls showed no significant change ($n = 6$). Furosemide was less effective. At $3.1 \pm 0.07 \times 10^{-3}$ *M* it produced an immediate reabsorption, which lasted 3 hr, but at $1.0 \pm 0.04 \times 10^{-4}$ *M* it increased secretion slightly ($n = 4$); controls showed no significant change ($n = 6$). The results are consistent with the presence of a chloride transport system, perhaps with sodium cotransport, as the major factor in fetal lung liquid secretion.

Fetal lungs produce large quantities of fluid, at a rate comparable to that of fetal urine production (1, 2). This fluid has three functions: (a) it helps support the fetus, since it forms part of the amniotic fluid; (b) it is necessary for the proper development of the lungs (3), and (c) it may be concerned in fetal salt and water regulation (4). Neither the cell source nor the exact mechanisms involved in fluid formation are known at this time. However, there have been suggestions (5, 8) that the alveolar type II cell may be the source of this fluid. At present, evidence from the composition of the fluid (6), from bidirectional ion fluxes (5), and from the intraalveolar negative (relative to plasma) electrical potential difference (5), all suggest that a major factor is a chloride transport system (7, 8), with possible cotransport of sodium (4). However, there are no reports of the effects of "loop" diuretics, such as bumetanide or furosemide, which are known to inhibit this type of transport system (9–11). The work reported here shows that both agents are able to inhibit lung liquid secretion, and supports the chloride-transport hypothesis of Olver and Strang (5) and Olver (8).

Materials and Methods. Thirty-four experiments were carried out on 20 fetal sheep, between 128 and 145 days of gestation (body weights 1.32–3.92 kg). All surgical procedures were carried out aseptically under halothane anesthesia. A loop of silicone tubing was inserted into the trachea (length of loop, 230 cm; internal diameter, 0.264 cm; volume 15.0 ml). At a flow rate of 22.9 ml/hr through the entire tubing, the pressure drop was less than 1 mm Hg. Between experiments, the loop allowed lung liquid to pass into the buccal cavity. During experiments, a T piece halfway along the loop was closed, so that fluid was diverted into a reservoir syringe; this allowed withdrawal of liquid directly from the lungs. Polyvinyl catheters were placed in (a) the fetal carotid artery and jugular vein, (b) the amniotic space, and (c) the maternal femoral artery and vein. Cannulae were tunneled under the maternal skin to a pouch on the flank. Five days were allowed for recovery from surgery.

The rate of secretion of lung fluid was measured by dye dilution techniques, as detailed elsewhere (4). The tracheal loop was clamped, and two impermeant tracers were added to the lung fluid (450 mg Dextran Blue 2000, Pharmacia, and 400,000–800,000 dpm ^{125}I-albumin). The ^{125}I-albumin was dialyzed for 12 hr before use, to remove traces of free iodide. Every 10 min after addition of tracers, lung liquid (20–40 ml) was withdrawn into a warm 50-ml reservoir syringe, and 0.5-ml samples were removed for analyses. Fluid re-

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maining in the reservoir was returned to the lungs. Mixing of fluid within the lungs was accomplished by withdrawal and return of fluid (20–30 ml) at 5- and 8-min intervals between samples. Pressure changes within the trachea were brief, usually around 5 cm H_2O and never above 15 cm H_2O; between mixing, the values were atmospheric. After addition, tracers were allowed to equilibrate for 1 hr. In the following hour, the resting secretion rate was estimated. After that hour, one of two procedures was carried out: (a) bumetanide (Roche; Nutley, N.J.) dissolved in ethanol:saline mixture (7:3) was added to lung fluid at 0.92×10^{-6}–4.64×10^{-4} *M*, final concentration; (b) furosemide (Lasix; Hoechst–Roussel, Somerville, N.J.) was added to the lung fluid at 0.9×10^{-4}–3.00×10^{-3} *M*, final concentration. Controls, with the appropriate carriers, were included in each type of experiment.

Rates of secretion of lung fluid were determined by the rate of dilution of dye (Beckman 25, spectrophotometer, at 620 nm) or isotope (LKB-Wallac 1282 Compugamma Universal Gammacounter). The total volume of lung fluid was calculated from the concentration of the two tracers, and the average from both methods was accepted as the final volume (except in studies of furosemide, where values from Dextran Blue were used). Rates of secretion were calculated from the slopes of the total volume of secretion against time, for 1-hr intervals (method of least squares), as detailed previously (4). Sequential allowances were made for all additions and withdrawals of fluid and tracer. Significance of a change in rate was estimated from changes in slope, using a *t* test for differences between two regressions. In addition, differences between mean values of secretion rates in successive hours, for similar groups of fetuses, were analyzed by one-way analysis of variance. Significance was accepted at $P < 0.05$. Where results from groups of fetuses were combined, volumes for each fetus were first expressed as a percentage of the volume present at the start of treatment, and then averaged together. Gestational ages were determined from tupping dates. Fetal weights were estimated according to Robillard and Weitzman (12). Fetal blood gases, pH, blood pressure, and heart rates were measured throughout the experiments.

The effect of bumetanide on lung liquid secretion. In 20 experiments on 11 chronic fetal sheep (128–145 days of gestation; 1.32–3.92 kg body wt), lung liquid production averaged 4.71 ± 0.61 ml/kg · hr. In 16 tests, bumetanide was added directly to the lung liquid. In the highest dose range ($1.03–4.64 \times 10^{-4}$ *M*), all six fetuses showed immediate reabsorption of liquid (average value in first hour, -2.67 ± 0.53 ml/kg · hr). Reabsorption continued in the second hour, although at a reduced rate. However, by the third hour a low secretion had returned (1.30 ± 0.87 ml/kg · hr; 72% below the initial rate). In the individual fetuses, all reductions in the first 2 hr, and 4 out of 5 in the final hour, were significant by regression analysis. This was confirmed for the first 2 hr after treatment by one-way analysis of variance of the mean values. Average changes for these fetuses, and for all other groups, are shown in Fig. 1.

Bumetanide was less effective in the lower range of $0.7–1.4 \times 10^{-5}$ *M*. It reduced secretion in all four tests, but never produced reabsorption. The average secretion rate in the first hour after treatment (1.89 ± 0.09 ml/kg · hr) was significantly reduced by 53.6%. All fetuses then started to recover, and were back to normal secretion by the final hour. The lowest dose range tested ($0.92–1.36 \times 10^{-6}$ *M*) gave variable effects. The two fetuses given the two highest doses showed a reduction in secretion, three fetuses gave no response, and the fetus with the lowest dose tested increased its secretion. Overall, there was a slight fall in secretion, but it was not significantly different from the control rate (Fig. 1).

When all responses were quantitated as the absolute fall or percentage fall in secretion rate during the first hour of treatment, there was a linear relationship with the logarithm of the dose of bumetanide (Fall in secretion rate (ml/kg · hr) = 17.63 + 2.83 log (dose of bumetanide); $r = 0.78$, $P < 0.001$). Six control experiments with ethanol–saline carrier showed no responses similar to bumetanide.

The effect of furosemide on lung liquid secretion. In 10 experiments on six chronic fetal sheep (132–143 days gestation; 2.51–3.73 kg body wt), lung liquid production averaged 3.73 ± 0.93 ml/kg · hr. In seven tests, furosemide was added to the lung liquid. In the highest dose range ($2.97–3.18 \times 10^{-3}$ *M*) all three fetuses showed immediate reabsorption of liquid

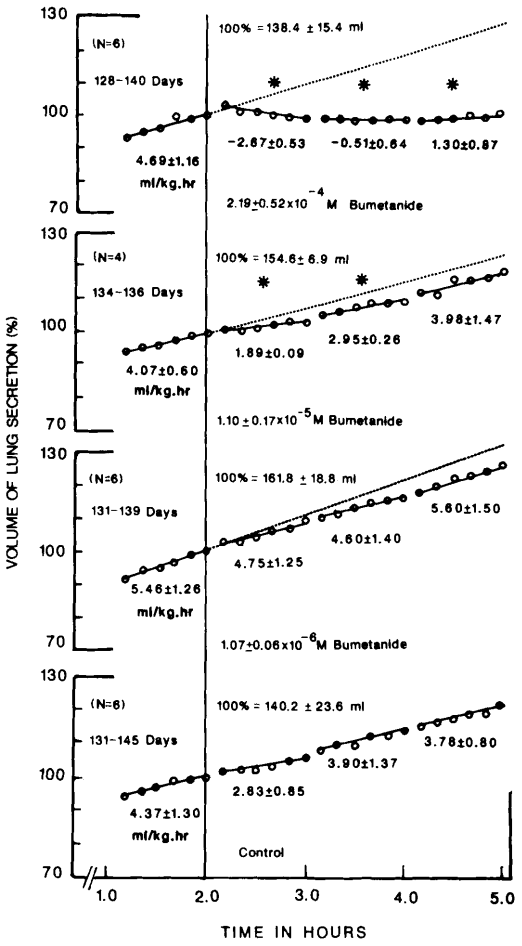

FIG. 1. The effects of injections of bumetanide into the lungs of fetal lambs on the production of lung liquid. The vertical line shows the time of injection of drug. The total volume of lung liquid secretion is expressed as a percentage of that present at injection of bumetanide. The actual volume for 100% is given at the top of each frame of the figure. The average concentration of bumetanide ± SEM is given at the bottom of each frame of the figure. Gestational age and number of animals used are presented to the left of each frame. Asterisks above lines show significant changes from original secretion slope (dotted line). *P* values are <0.05. Numbers below lines are average rates of secretion in ml/kg · hr ± SEM.

(average value, in the first hour, −7.22 ± 2.20 ml/kg · hr). Reabsorption continued throughout the second and third hours, in all fetuses (average values, −4.75 ± 1.17 and −2.59 ± 1.04 ml/kg · hr, respectively). In individual fetuses, significant reductions were present in all three hours following treatment. Average changes for these fetuses, and all other groups, are shown in Fig. 2.

In four tests, furosemide in the lower range of 0.9–1.1 × 10^{-4} *M* never produced a significant reduction in secretion at any time. On the average, there was a steady increase in secretion rate (Fig. 2). Controls showed no significant change at any time (Fig. 2).

Blood gases, pH, Blood pressure, and heart

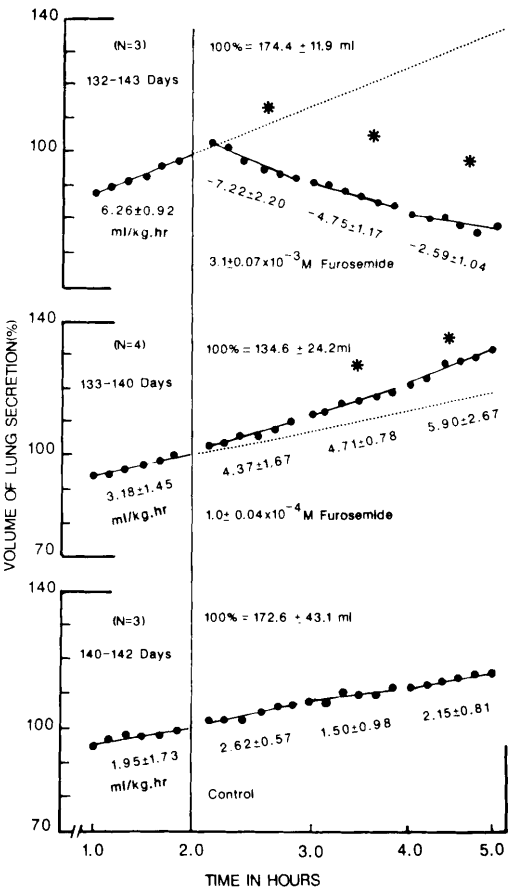

FIG. 2. The effects of injections of furosemide into the lungs of fetal lambs on the production of lung liquid. The vertical line shows the time of injection of drug. The total volume of lung liquid secretion is expressed as a percentage of that present at injection of furosemide. The actual volume for 100% is given at the top of each frame of the figure. The average concentration of furosemide ± SEM is given at the bottom of each frame of the figure. Gestational age and number of animals used are indicated to the left of each frame. Asterisks above lines show significant changes form original secretion slope (dotted line). *P* values are <0.05. Numbers below lines are average rates of secretion in ml/kg · hr ± SEM.

rate. There were no statistically significant changes in blood gases, pH, blood pressure or heart rate between control and treated groups. Thus values were averaged and are presented as such: $PO_2 = 23.9 \pm 3.0$ (SD)., $PCO_2 = 52.3 \pm 5.5$ (SD); pH = 7.35 ± 0.04 (SD); systolic blood pressure = 63.6 ± 6.87 (SD); diastolic blood pressure = 38.6 ± 5.6 (SD); heart rate = 171 ± 29 (SD). Vascular pressures were referred to the midchest position of the fetal animals. Blood gases and pH were corrected to fetal body temperature.

Discussion. These results show that bumetanide is capable of inhibiting lung liquid secretion and may, in fact, promote reabsorption. When placed in the luminal compartment at $2.19 \pm 0.52 \times 10^{-4}$ *M*, the drug caused reabsorption, and at $1.10 \pm 0.17 \times 10^{-5}$ *M* it inhibited secretion. Below this level, at $1.07 \pm 0.06 \times 10^{-6}$ *M*, it had no consistent effect. Furosemide, given at $1.0 \pm 0.04 \times 10^{-4}$ *M*, a level similar to the most effective dose of bumetanide, did not reduce secretion. However, when the level was raised to 14 times that of the highest dose of bumetanide, it caused a marked reabsorption. Since both these agents are thought to work on a chloride transporter system (9–11), these data support the current hypothesis of Olver and Strang (5) and Olver (7, 8) that lung liquid secretion is based on a chloride-transport system, which operates from the blood to the lumen of the lung. The relative activity of the two diuretics is similar to their activity in inhibition of chloride transport in the isolated renal tubule, where bumetanide is 14 times more potent than furosemide (9). However, these drugs have effects other than those on chloride transport, notably a weaker inhibition of carbonic anhydrase activity (13). Nevertheless, it is unlikely that the effects of bumetanide on lung secretion are due to this side effect, since the affinity of the drug for this system is too low (13). In addition, if the effects on lung secretion were due to carbonic anhydrase inhibition, the relative activities of the two diuretics would be reversed, since furosemide is seven times more active than bumetanide on this enzyme. At present, it is difficult to explain the increase in secretion which occurred with low doses of furosemide (10^{-4} *M*), and with the lowest dose of bumetanide (0.92×10^{-6} *M*). This might indicate the presence of a more sensitive chloride transporting system, operating at a lower level, from alveolar space to blood. This weaker system, working in opposition to the major secretion, might be "unmasked" at birth.

For many years, loop diuretic drugs have been used to treat pulmonary edema (14, 15); however, their mechanism has not been clear. Bland and his associates (16) have evaluated the effects of furosemide on pulmonary transvascular fluid filtration in conscious newborn lambs. They concluded that the major effect was indirect, by way of the kidneys. Furosemide produced a diuresis which reduced lung vascular hydraulic pressure, so that the protein osmotic pressure of the blood could reduce the movement of fluid toward the alveoli more effectively. However, our studies suggest other possible mechanisms. Although the work deals with the fetus *in utero,* it is possible that secretion persists in the newborn in certain cases ("wet lung"). This secretion might be reduced directly by furosemide, but only at very high doses. This is in agreement with several recent studies (17–19) showing the ineffectiveness of furosemide in treating infant respiratory distress syndrome. However, the work given here suggests that bumetanide might be more effective. Although most of our studies have tested the effects of bumetanide given directly to the pulmonary epithelium, at known concentrations, preliminary studies show the intravenous infusion of the drug is also effective. Therefore, the work presented here suggests that bumetanide might be useful in reducing neonatal secretions, and that the effect is probably an inhibition of chloride transport mechanisms.

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