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vascular permeability

Studies on Group B β-Hemolytic *Streptococcus*. II. Effects on Pulmonary Hemodynamics and Vascular Permeability in Unanesthetized Sheep

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Summary

To study the effects of Group B β-hemolytic *Streptococcus* on the pulmonary circulation and lung fluid balance, live and heat-killed bacteria and their toxin were infused into an awake sheep lung-lymph preparation. In every case, the response was biphasic; there was an initial period of marked pulmonary hypertension and high flow of protein-poor lymph associated with tachypnea, chills, and fever. A second phase followed during which pulmonary vascular pressures returned to near baseline and remained stable, but lymph flow remained high. The changes seen in the initial phase resemble the previously reported response to mechanically increased pulmonary vascular pressure and suggest that the increase in fluid filtration is secondary to increased microvascular pressure. During the second phase after toxin infusion, the increase in lung lymph flow was paralleled by an increase in lymph protein clearance. This cannot be accounted for by the hemodynamic changes alone and suggests that the permeability of lung microvascular walls to protein was increased. It is concluded that group B β-hemolytic streptococcal toxin in the sheep model causes pulmonary hypertension and increased pulmonary vascular permeability.

Speculation

The effects caused by group G β-hemolytic *Streptococcus* toxin on the pulmonary circulation may be relevant to the pathogenesis of the respiratory distress and shock seen in newborns with this infection. Further understanding of the effects of this toxin could provide means for therapeutic intervention.

Group B β-hemolytic *Streptococci* have been implicated in fatal human disease since 1938 (15) and are currently the most frequent pathogens associated with neonatal infectious mortality (19, 20), causing one death per 1000 live births (14). In 1973, Franciosi *et al.* (14) observed two distinct clinical syndromes in newborns, based upon the time of onset after delivery: early onset disease, usually appearing within a few hours of birth, heralded by apnea and characterized by respiratory distress and shock, with a fatality rate over 50% (1, 25), and late onset disease with a lower mortality rate, 14 to 18% (1, 33), commonly presenting after a week of age and characterized by meningeal invasion. Early onset disease has definite similarities to gram-negative endotoxemia (13).

Gram-negative endotoxins increase permeability of pulmonary microvessels (6, 10), and respiratory distress in patients with gram-negative sepsis has been attributed to this change. Katzenstein *et al.* (21) found pulmonary congestion and hyaline membranes, usually without pneumonia, at autopsy in newborns dying with group B streptococcal sepsis and postulated damage to the capillary endothelium as a possible mechanism.

The purpose of this investigation was to determine the effects of group B β-hemolytic streptococci, type III, on pulmonary hemodynamics and pulmonary vascular permeability of young adult sheep. Live and heat-killed organisms and a toxin produced by the same organism (18), which appears to have endotoxin-like properties, were studied.

MATERIALS AND METHODS

A chronic sheep lung-lymph preparation which has been described in the literature was used (5, 7–9). Through bilateral thoracotomies in yearling sheep (35 to 45 kg), catheters were placed directly into the main pulmonary artery, left atrium, and the efferent duct from the caudal mediastinal lymph node. The tail of the node was resected to eliminate nonpulmonary lymph, and catheters were placed in the right atrium and thoracic aorta through neck vessels. Lymph collected from animals prepared in this way is primarily from the lung because lymph flow increases when pulmonary vascular pressure is increased mechanically, but lymph flow does not increase when systemic venous pressure is increased (10, 11).

All experiments were done with the sheep unanesthetized, standing unrestrained in a cage. Vascular pressures were measured continuously with pressure transducers (Statham P23Gb; Gould-Statham Instruments, Inc., Hato Rey, Puerto Rico) and an electronic recorder (Hewlett-Packard Co., Palo Alto, CA). Lymph flow was measured at 15-min intervals by recording the volume drained into a graduated tube. Protein concentration was measured in plasma from blood drawn each hour and in lymph pooled at 30-min intervals using an automated system (AutoAnalyzer; Technicon Instrument Corp., Tarrytown, NY) by a modified Biuret method (12). Arterial blood gases were measured every 30 min in a pH-Blood Gas Analyzer (Model 213; Instrumentation Laboratory, Inc., Lexington, MA), and rectal temperature was monitored continuously with a YSI tele-thermometer (Yellow Springs Instrument Co., Yellow Springs, OH).

Group B β-hemolytic *Streptococci* type III were used for all experiments. The strain was isolated from a baby who died from early onset disease at Vanderbilt University Hospital Intensive Care Nursery. Grouping and typing were done by the fluorescent antibody technique (23, 30) with antisera kindly provided by Dr. Edward O. Mason (Baylor College of Medicine).

Bacteria for each experiment were obtained from the overnight growth (18 hr) of five sheep blood agar plates and five trypticase soy broth tubes. The organisms were suspended in 100 ml of 0.9% NaCl solution, and the number of organisms was determined by serial dilution plates. This suspension was used unaltered for the live organism infusion and was boiled in a water bath for 1 hr for the heat-killed organism infusion. All infusates were checked for purity and sterility.

Fractionation of the bacteria and isolation of a "native" toxin are described elsewhere (18). Suspension cultures of the same streptococcal strain which was used in whole organism experi-

ments were grown for 22 hr using modified Todd-Hewitt broth (2). The cells were centrifuged and discarded. The supernatant was precipitated with ethyl alcohol and purified by ion exchange chromatography and gel filtration.

The dose of the toxin used for all infusions was 2.2 mg, suspended in 80 ml of 0.9% NaCl solution. Control infusions of bacteria-free media processed in the same fashion have been previously described (18, 29).

Lung-lymph protein clearance was calculated as the product of lymph flow and lymph/plasma total protein concentration, and total protein permeability-surface area products (PS) were estimated by the equation of Renkin (26):

$$PS = \frac{LR}{1\text{-}R} \quad (1)$$

where L is lymph flow and R is the lymph/plasma concentration ratio for protein. The calculation assumes that transvascular protein movement is only by diffusion, although it has been suggested by several workers (10, 17, 22) that a substantial fraction of transvascular protein movement is convective rather than diffusive.

Pulmonary microvascular pressure (Pmv) was estimated using the formula

$$Pmv = Pla + 0.4\ (Ppa - Pla) \quad (2)$$

where Pla and Ppa are the mean left atrial and pulmonary artery pressures, respectively, and 0.4 is the fraction of total pulmonary vascular resistance assumed to be downstream from the microvascular exchange surface (16, 31).

All statistical calculations to compare values before and after infusions were done using a two-tailed paired t test. A P value of less than 0.05 was considered significant.

RESULTS

LIVE ORGANISMS

Five infusions of 6×10^{10} to 2.9×10^{11} organisms were done in four sheep. The response varied in intensity, but followed a consistent pattern, which is illustrated in Figure 1. Five to 15 min after starting the infusion, the sheep developed tachypnea, chills, and fever; pulmonary artery pressure rose, and left atrial pressure remained unchanged (phase 1). After 30 to 45 min, pulmonary artery pressure returned to near baseline but remained slightly elevated for 4 to 6 hr (phase 2). Lymph flow increased during the initial phase and stayed higher than baseline in the second phase. Lymph protein concentration fell in phase 1 and remained lower than baseline during the second phase. Although lymph flow was higher in the second phase, lung lymph protein clearance did not increase significantly (Fig. 2). Rectal temperature rose 1.15 ± 0.2°C ($P < 0.05$) during phase 1 and remained elevated for 60 to 90 min. Arterial pH did not change significantly in either phase. Arterial Po_2 decreased 9 ± 3 torr ($P < 0.05$) during the pulmonary arterial pressure rise and returned to baseline values in phase 2.

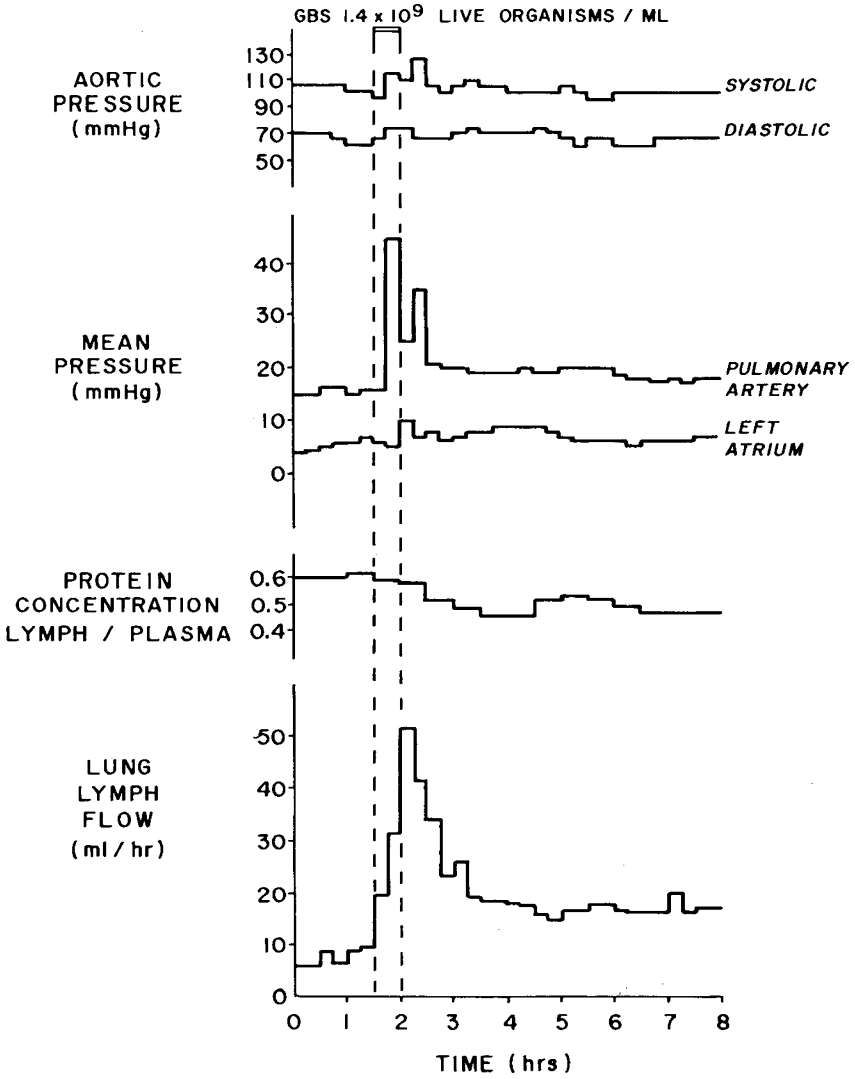

Fig. 1. Responses of vascular pressures, lymph flow, and protein concentration to the infusion of live organisms in a sheep.

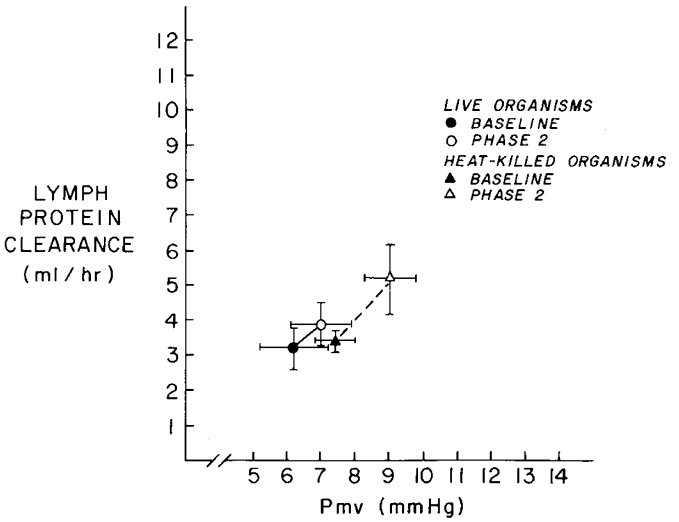

Fig. 2. Average steady-state lymph protein clearance as a function of estimated microvascular pressure (*Pmv*) for five live organisms infusions and five heat-killed organisms infusions. *Bars*, mean ± SE.

Arterial P_{CO_2} did not change. Mean values for the five experiments are summarized in Tables 1 and 2.

Figure 3 shows the relationship between lymph/plasma protein ratio and lymph flow during phase 2; the regression line and confidence limits for previously reported studies with increased pressure are also shown (3, 8). The values with live bacteria are very similar to those for mechanically increased pressure. Total protein permeability-surface area product did not change significantly in phase 2 of the reaction (Table 1).

HEAT-KILLED ORGANISMS

Five infusions of 8×10^7 to 3.5×10^{10} organisms were done in five sheep. In every instance, the control cultures were negative after boiling. The response was identical to that obtained with live organisms. There was an initial phase of pulmonary hypertension and high flow of protein-poor lymph associated with chills and a rise in rectal temperature of 0.9 ± 0.2°C ($P < 0.05$). A second phase followed where pulmonary artery pressure returned to near baseline values, but lymph flow remained high. Mean values for the five experiments are summarized in Tables 1 and 2.

Neither lung lymph protein clearance (Fig. 2) nor total protein permeability-surface area product (Table 1) were increased significantly.

The relationship between lymph/plasma protein ratio and lymph flow during the second phase was similar to that of the mechanically increased pressure studies (Fig. 3).

TOXIN

Five infusions of the toxin (53 to 65 μg/kg) were done in three sheep. A typical response is illustrated in Figure 4. As with the whole organisms, the response was biphasic, but there were clear differences. The first phase was delayed, beginning 30 to 45 min after starting the infusion. It was characterized by tachypnea, chills, fever, pulmonary hypertension, and high flow of protein-poor lymph lasting for 45 to 60 min. Subsequently, the animal appeared well, pulmonary artery pressure was slightly above baseline and stable, lymph flow was 1.7 times the baseline value, and the ratio of protein concentration in lymph to that in plasma increased and remained high for 6 to 8 hr. The data for both phases in all experiments are summarized in Table 1.

Toxin infusion caused rectal temperature to increase from 39.3 ± 0.06°C to 40.2 ± 0.17°C ($P < 0.02$) and arterial P_{O_2} to decrease 25 ± 7 torr ($P < 0.05$) during the initial response. Arterial P_{CO_2}

Table 2. *Summary of arterial blood gas data for all experiments.*

Experiment	Pa_{O_2} (torr)	Pa_{CO_2} (torr)	pH
Live bacteria ($n = 5$)			
Baseline	83 ± 3[1]	31 ± 3	7.50 ± 0.01
Phase I	75 ± 6[2]	30 ± 4	7.51 ± 0.01
Phase II	85 ± 5	29 ± 3	7.54 ± 0.03
Heat-killed bacteria ($n = 5$)			
Baseline	94 ± 5	30 ± 2	7.50 ± 0.01
Phase I	85 ± 2[2]	32 ± 2	7.50 ± 0.01
Phase II	92 ± 4	30 ± 2	7.54 ± 0.01
Toxin ($n = 5$)			
Baseline	94 ± 3	31 ± 1	7.51 ± 0.02
Phase I	69 ± 6[2]	35 ± 1	7.46 ± 0.03
Phase II	93 ± 3	29 ± 1	7.54 ± 0.01

[1] Mean ± S.E.
[2] $P < 0.05$.

Table 1. *Summary of pressures and lymph data for all experiments*

Experiment	Mean Pressure (mm Hg)		Lymph flow (ml/hr)	Protein concentration (g/100 ml)			PS[1] (ml/hr)
	Pulmonary artery	Left atrium		Lymph	Plasma	Lymph / Plasma	
Live bacteria ($n = 5$)							
Baseline	17 ± 0.8[2]	−1.5 ± 1.1	4.7 ± 0.7	3.9 ± 0.2	6.1 ± 0.3	0.65 ± 0.03	9.8 ± 2.9
Phase 1	30 ± 3.3[3]	−2.7 ± 0.7	10 ± 1.0[3]	3.4 ± 0.2[3]	6.0 ± 0.3	0.57 ± 0.04[3]	
Phase 2	21 ± 2.7	−2.7 ± 1.4	6.5 ± 0.5[3]	3.6 ± 0.2[3]	5.9 ± 0.3	0.60 ± 0.04[3]	11.2 ± 2.9
Heat-killed bacteria ($n = 5$)							
Baseline	16 ± 0.7	1.8 ± 1.0	5.6 ± 0.7	3.5 ± 0.3	5.6 ± 0.3	0.63 ± 0.01	9.5 ± 0.9
Phase 1	37 ± 4.2[3]	2.3 ± 2.3	26 ± 7.7[3]	2.6 ± 0.3[3]	5.6 ± 0.4	0.47 ± 0.03[3]	
Phase 2	19 ± 1.1	2.6 ± 1.2	9.3 ± 2	3.3 ± 0.4	5.5 ± 0.4	0.59 ± 0.03	12.8 ± 1.9
Toxin ($n = 5$)							
Baseline	17 ± 0.7	4.5 ± 1.1	6.7 ± 0.8	3.9 ± 0.3	6.0 ± 0.5	0.67 ± 0.02	14.5 ± 3.1
Phase 1	46 ± 3.2[3]	1.7 ± 1.1	33 ± 3.6[3]	3.1 ± 0.2[3]	6.0 ± 0.4	0.52 ± 0.02[3]	
Phase 2	21 ± 0.3[3]	4.8 ± 0.8	11.3 ± 0.9[3]	4.0 ± 0.1	6.0 ± 0.4	0.68 ± 0.03	27.3 ± 4.6[3]

[1] PS, permeability-surface area product.
[2] Mean ± S.E.
[3] $P < 0.05$.

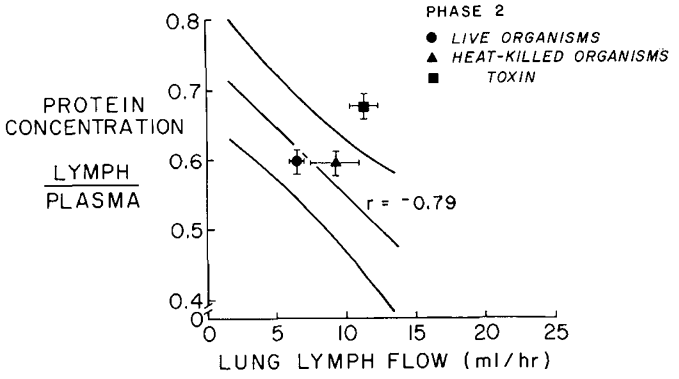

Fig. 3. Average steady-state lymph/plasma protein concentration as a function of lung lymph flow for five live organisms, five heat-killed organisms, and five toxin infusions. The regression line and 95% confidence limits are for reported studies where lung vascular pressures were increased mechanically (3, 8). *Bars*, mean ± SE.

and pH did not change significantly in either phase. The blood gas data are summarized in Table 2. Total protein permeability-surface area product and lung lymph protein clearance increased significantly during the steady-state second phase of the reaction, even though pulmonary vascular pressures were only slightly elevated (Fig. 5).

Figure 3 shows the relationship between lymph/plasma total protein concentration ratio and lymph flow during the second phase of the toxin infusions, which is different from the values obtained with the mechanically increased pressure studies.

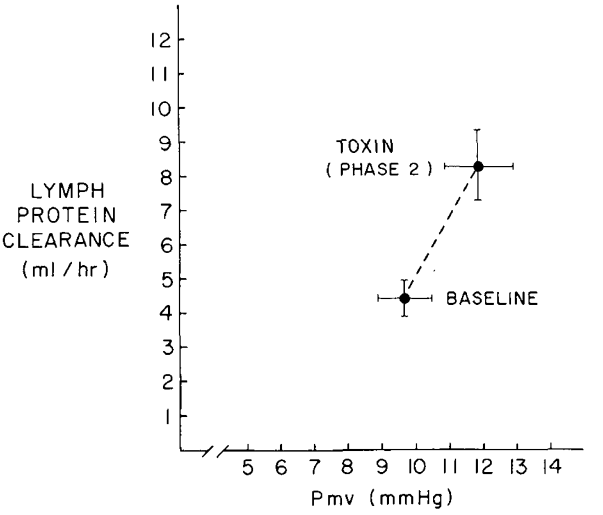

Fig. 5. Average steady-state lymph protein clearance as a function of estimated microvascular pressure (*Pmv*) for five toxin infusions. *Bars*, mean ± SE.

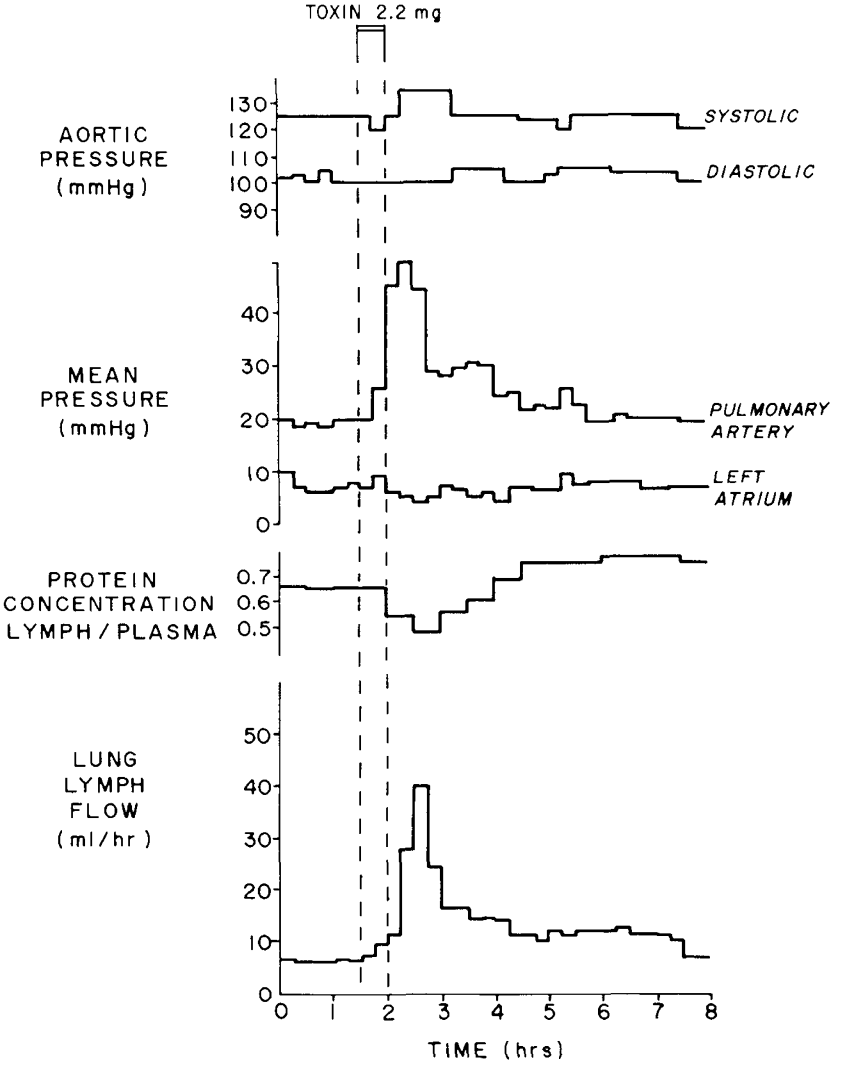

Fig. 4. Responses of vascular pressures, lymph flow, and protein concentration to the infusion of 2.2 mg of toxin in a sheep.

DISCUSSION

A response to intravenous infusion of group B β-hemolytic *Streptococci* type III and to a toxin from the same organism has been demonstrated in chronically instrumented, unanesthetized sheep. This response is similar to that obtained by the infusion of whole *Pseudomonas* bacteria or *Escherichia coli* endotoxin (6, 10). In each case, there is an initial period of marked pulmonary hypertension and a later period of high lung lymph flow when pulmonary vascular pressures are slightly elevated and stable.

Although the magnitude of pressure and lymph flow response differed among animals, the initial phase was qualitatively similar in all studies. The increase in lung lymph flow with decreased lymph to plasma protein concentration ratios is similar to the previously reported response to mechanically increased lung vascular pressures (11), and suggests that the increased fluid filtration from lung microvessels during this phase is primarily due to increased microvascular pressures. The earlier onset of pulmonary hypertension in the whole organism infusions could be due to microembolization of clumps of bacteria resulting in an earlier rise of the pulmonary artery pressure (32) or to substances other than the toxin present in the bacteria. This question cannot be answered from the available data.

During the second phase, the responses to whole organisms and to the toxin were different. Although in all three groups a period of apparent steady state developed when the lymph flow remained above baseline values, the lymph/plasma total protein concentration ratio increased in the toxin group, resulting in a much larger increase in lymph protein clearance. The toxin effects on lung lymph flow and protein transport cannot be explained by hemodynamic changes alone.

Under steady-state conditions, lymph flow from an organ reflects net fluid filtration from the organ's exchanging vessels (17). In addition, lymph protein content does not change during transit across lymph nodes or through peripheral lymphatics (24), and steady-state lymph protein concentration has been thought to represent protein concentration in net microvascular filtrate (17). If these assumptions are true, then the streptococcal toxin increased net transvascular fluid and protein movement in the lung out of proportion to its effects on pulmonary vascular pressures; the permeability of the microvascular walls to protein was increased during the late phase of the reaction. This conclusion is corroborated by the relationship between lymph/plasma protein concentration and lung lymph flow (Fig. 3). These effects are similar to those obtained with *Pseudomonas* bacteremia and *E. coli* endotoxin infusion, which may suggest similar pathogenetic mechanisms (10).

The fact that lung vascular permeability did not increase with the live or heat-killed bacteria may be because the amount of toxin bound to organisms is small compared to the amount in the medium, so that the total toxin dose may have been much smaller in the whole organism studies.

The delayed appearance of both phases of the response from the time of the beginning of the toxin infusion suggests that the changes may not be a direct effect, but rather that they are mediated by endogenous vasoactive substances. There are several known mediators that could be involved (4, 8, 9), but further studies will be necessary before any rational suggestion can be made. Whether the same biochemical mechanisms are involved in both phases of the reaction and whether the two phases are independent or interdependent also remains to be established.

Increased permeability of the lung microvessels has been thought to be the cause of the respiratory distress that develops with gram-negative endotoxemia (27, 28), and it is tempting to postulate a similar abnormality to account for the respiratory distress seen in babies with early onset group B streptococcal septicemia. Although there are data suggesting that babies with this disease may have circulating bacterial products with endotoxin-like properties (13), such a product has not been isolated from infected infants.

The data presented in this paper support the idea that group B β-hemolytic streptococci type III are capable of producing a toxin that, at least in sheep, causes pulmonary hypertension and increased lung vascular permeability. These effects are similar to those seen with gram-negative endotoxins and may be relevant to the pathogenesis of the respiratory distress seen in newborns with group B streptococcal sepsis.

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