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glycerol pulmonary phosphatidylcholine
lung

The Significance of Circulating Glycerol as a Precursor of Pulmonary Phosphatidylcholine in the Developing Mammalian Lung

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Extract

There is scant information regarding the contribution made by circulating precursors to pulmonary phosphatidylcholine synthesis in the developing mammalian lung. *In situ* pulmonary artery perfusions were performed in term New Zealand newborn rabbits with physiologic buffer containing either 3.6 mM or 10.8 mM glycerol. There was a twofold increase in nanomoles of glycerol-phosphatidylcholine synthesized at 30 min when the higher concentration of glycerol was used. Continuing with the higher concentration, a near three-fold increase was observed between the 30-min and 60-min perfusions. This data indicates that the *de novo* synthesis of pulmonary phosphatidylcholine is influenced by the concentration of glycerol in the perfusate as well as the duration of perfusion.

Speculation

The observation that the concentration of circulating glycerol can influence the *de novo* synthesis of pulmonary phosphatidylcholine suggests that glycerol may also play a role in providing precursor for pulmonary surfactant synthesis. The biochemical similarity of lipid metabolism at birth between human newborn infants and the newborn rabbit encourages extrapolation of this data to humans. The question is raised as to the influence that intravenous glycerol at physiologic concentration would have on pulmonary phosphatidylcholine synthesis in the infant with hyaline membrane disease.

Scant information is available regarding circulating precursors used for pulmonary surfactant synthesis in the developing mammalian lung. Naimark (6) reports that the normal lung's *de novo* lipid synthesis is not limited by availability of circulating substrate. Godinez *et al.* (2) observed, however, increased pulmonary incorporation of [1-^{14}C]palmitate with increased medium palmitate concentration. They reported decreased [1-^{14}C]palmitate incorporation when oleate was added to the medium, suggesting that fatty acid incorporation into phospholipid was related to the total fatty acid concentration in the medium. Scholz and Rhodes (7) reported that rats decreased the *in vitro* utilization of glucose for pulmonary phospholipid fatty acid synthesis during starvation as compared with the fed state. This suggested that during starvation, pulmonary phospholipid fatty acid synthesis relied on circulating fatty acid rather than *de novo* synthesis.

Recent *in vitro* and morphologic data (4, 5), however, strongly suggests that circulating glycerol could be used for the

de novo synthesis of pulmonary phosphatidylcholine (PC) and thus contribute to pulmonary alveolar stability. Pulmonary perfusion studies with physiologic buffer containing varying concentrations of glycerol demonstrate the influence that circulating glycerol has on pulmonary PC synthesis.

MATERIALS AND METHODS

Females purebred New Zealand White rabbits (9) were mated with a single buck under direct visualization. Mating was considered zero time of gestation. Twenty-nine to 30-day rabbit fetuses were delivered by caesarean section immediately after killing the doe with 20–30 ml air via an ear vein. The rabbits were allowed to breathe room air for 2 hr in a warmed, wooden brooder (33–35°). A thick, waxed ligature was then tied around the neck of each pup, and the animal was immediately submerged in ice until gasping ceased (2–4 min). The heart was exposed by careful dissection, leaving the pleural deflection of the mediastinum intact. A no. 20 polyethylene tube was threaded into the pulmonary artery via an incision in the right ventricle. The left ventricle was then incised to prevent pulmonary edema and the lungs perfused with oxygenated Krebs-Ringer bicarbonate buffer containing 10 μCi [2-^{3}H]glycerol (specific radioactivity 200 mCi/mmol) and cold glycerol either 3.6 mM or 10.8 mM and 1.0 mg glucose/ml buffer. The rate of perfusion was 2.5–3.0 ml/min. A total of 52 perfusions were done: 13 for 30 min, 20 for 60 min and 19 for 180 min. Lung lipids were extracted and analyzed for radioactivity as described previously (4). A sample of each lung was taken for DNA analysis by the method of Burton (1). Significance was determined using Student's t test.

RESULTS

Figure 1 shows the mean nanomoles of glycerol-lipid per milligram of DNA in the lung after perfusion with glycerol-buffer solution containing either 10.8 mM glycerol or 3.6 mM glycerol. At 30 min there is a significant difference ($P < 0.01$) in synthesized glycerol lipid when the concentration of glycerol is varied in the perfusate. This significance, however, is lost at 60 min. Using the higher concentration, nearly a three fold increase in glycerol lipid is seen occurring between 30 and 60 min at a significance level of $P < 0.05$. Although an increase in glycerol lipid was observed by extending the more concentrated perfusate to 180 min, the difference was not significant ($P < 0.20$). This leveling off of incorporation after 60 min has also been observed in the *in vitro* studies reported previously (5).

Having demonstrated that total glycerol-lipid synthesis could be influenced by varying the perfusate concentration of glycerol and the duration of the perfusion, the various lipid classes were analyzed with particular emphasis on PC and its immediate precursor, diglyceride. No information is available as to the fatty acid side chains in any of these experiments.

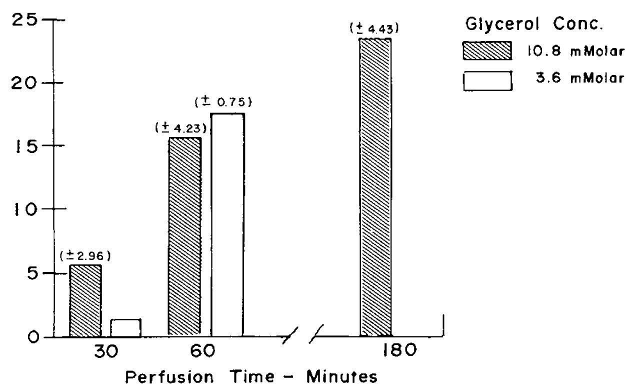

Fig. 1. The mean nanomoles of pulmonary glycerol-lipid per milligram of lung DNA synthesized during timed perfusion with glycerol-buffer perfusate of varying concentrations and containing tracer 2-[^{3}H]glycerol (mean ± SD).

Figure 2 demonstrates the mean [2-^{3}H]glycerol-PC per milligram of lung DNA as related to variations in glycerol concentration and duration of perfusion. Other phospholipids are not shown. Pulmonary PC, however, accounted for 60–75% of all radioactivity. The difference seen after 3.6 mM and 10.8 mM glycerol concentration at 30 min is significant at $P < 0.05$. No significant difference is seen at 60 min. The difference observed between 30 and 60 min with 10.8 mM concentration glycerol is not significant ($P < 0.4$). The difference between 30 and 60 min with 3.6 mM glycerol is highly significant ($P < 0.001$). These findings would suggest that pulmonary PC synthesis from glycerol reaches its maximum early with the higher concentrations, whereas at lower glycerol concentration, significant incorporation continues for an additional 30 min.

Various glycerol lipids (di- and triglycerides) were analyzed from the 30-, 60-, and 180-min perfusions using the 10.8 mM glycerol. Incorporation was essentially the same for all time periods. Nearly two-thirds of the label incorporated for all time periods was found in diglyceride. Here again, the perfusion studies support *in vitro* findings that diglyceride, an immediate precursor for phosphatidylcholine, is the glyceride most actively synthesized from available circulating glycerol.

DISCUSSION

Blood glycerol rises in the immediate newborn adaptive period, with serum blood levels found in excess of 0.3 mM (3). This rise apparently reflects postnatal lipolysis. The term human neonate is capable of utilizing as much as 1.5 mmol glycerol/hr, presumably for gluconeogenesis (8). Evidence from this laboratory suggested that circulating glycerol may also be an important precursor for pulmonary PC synthesis by the developing mammalian lung (4, 5). The previous *in vitro* and morphologic observations are supported by the perfusion data presented in this report. The lower concentration (3.6 mM) used in our perfusions, complements that used by Wolf *et al.* (3.5 mM) (8). The higher concentration (10.8 mM) represented an increase we felt necessary to impart change in the system. This report suggests that the concentration of circulating glycerol and the duration of perfusion may indeed influence the rate of pulmonary PC synthesis in the developing mammalian lung.

SUMMARY

In situ, timed pulmonary artery perfusions indicate that the newborn rabbit lung utilizes circulating glycerol for phosphatidylcholine synthesis. Variation in the glycerol concentration from 3.6 mM to 10.8 mM increased pulmonary-synthesized

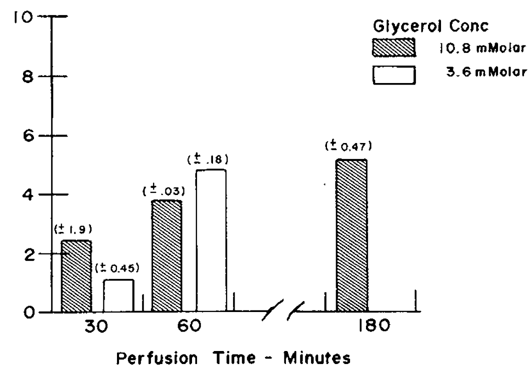

Fig. 2. Pulmonary phosphatidylcholine synthesis as a function of perfusion time and glycerol concentration (mean ± SD).

glycerol-phosphatidylcholine nearly twofold during a 30-min perfusion. Continuing the perfusions with 10.8 mM glycerol concentration for a 60-min perfusion resulted in nearly a threefold increase in synthesized glycerol-phosphatidylcholine. This data supports our previous observations that circulating glycerol may be an important precursor for pulmonary phosphatidylcholine synthesis.

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Coagulant, endotoxin, L-epinephrine, leukocytes, prostaglandin E_1, umbilical cord

Studies on Tissue Factor Activity and Production by Leukocytes of Human Umbilical Cord and Adult Origin

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Extract

Human adult and umbilical cord-derived leukocytes were shown to be capable of generating tissue factor activity on exposure to endotoxin and to reduced pH. Blood for leukocyte separation was collected from normal adults and from newly delivered sections of umbilical cord and mixed leukocyte preparations were obtained by separation over methyl cellulose Hypaque. The coagulant activity of the cell suspension was assayed using a one-stage or two-stage method. Cord-derived leukocytes were shown to develop greater coagulant activity than adult-derived leukocytes when stimulated by endotoxin *in vitro* at 2,000 cells/mm^3. This response to endotoxin was partially inhibited by prior exposure of the cells to prostaglandin (PG) E_1 and to L-epinephrine. Acetylcholine stimulated the production of coagulant activity in the absence of endotoxin. Both cord and adult-derived leukocytes (20,000/mm^3) developed coagulant activity when exposed to pH reduction by lactic or hydrochloric acids and this activity was shown to be tissue factor.

Speculation

TF production by leukocytes (monocytes) after endotoxin stimulation may explain the frequently encountered association of disseminated intravascular coagulation (DIC) with gram-negative septicemia in the newborn infant. The effect of pH reduction in association with hypoxia in the perinatal period, by causing tissue factor (TF) activity to become available, may be the trigger mechanism for the coagulation changes of varying degrees of DIC seen in these situations. Necrotizing enterocolitis could be the result of endotoxin penetration of the bowel mucosa which induces monocyte chemotaxis and local TF production by these cells; this would cause local intravascular coagulation with platelet consumption and necrotic sequelae. It remains to be established whether inhibition of *in vivo* endotoxin-induced monocyte TF production can be achieved with PGE_1, and whether such therapy might improve the outcome in gram-negative septicemia.

Evidence of the involvement of leukocytes in blood coagulation has accumulated from several sources; reports have documented the associations between DIC and promyelocytic leukemias (9, 13, 27), and investigations on the Sanarelli-Shwartzman reaction (SSR) (10, 30, 33) have shown leukocytes to be essential for the full expression of the reaction.

Evidence more directly linking a mechanism for the initiation of coagulation with leukocytes has come from studies on peritoneally derived rabbit leukocytes (20). These