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EVIDENCE FOR LACTATE UTILIZATION FOR FETAL LUNG GLYCOGEN SYNTHESIS

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Fetal rabbit lungs from 23 day gestation animals were used to investigate the potential role of lactate as a substrate for fetal lung glycogen synthesis. Fetal lactate dehydrogenase activity was approximately twice that found in the adult lung, while the activity of phosphoenolpyruvate carboxykinase was elevated fourfold over the adult value. Pyruvate carboxylase activities were similar in both fetal and adult lungs. Studies employing fetal lung explants in organ culture indicated that the presence of both glucose and lactate may be necessary for glycogen accumulation in the developing fetal lung. These data support the hypothesis that lactate is an important precursor for fetal lung glycogen.

The last 10% of gestation in mammals is characterized by a surge in the production of surfactant phospholipids in the fetal lung (1,2). Temporally related to this increased production is a rapid decline in pulmonary glycogen stores. This inverse relationship between surfactant and glycogen concentrations has led to the hypothesis that glucose derived from glycogen is an important substrate for fetal lung phospholipid synthesis (3,4). Studies by Bourbon, et al. (5), Farrell and Bourbon (6), and Chi (7), lend credence to this hypothesis. Glycogen may therefore be a significant component for the maturation of the pulmonary surfactant system.

Despite the potential importance of glycogen, very little is currently known about the substrate or hormonal requirements for fetal lung glycogen synthesis. Lactate, which is now thought to be a major substrate for adult liver glycogen (8), is present in the fetal circulation in relatively high concentrations (8-12 mM) (9,10). Glucose levels, on the other hand, are generally lower than those found in the adult animal (9,10). This high lactate-low glucose environment might indicate that lactate is an important carbohydrate resource for fetal lung glycogen production. The studies reported here were undertaken to investigate the role of lactate in fetal lung glycogen accumulation. Several enzymes necessary for the conversion of lactate to glycogen, namely, lactate dehydrogenase, phosphoenolpyruvate carboxykinase, and pyruvate carboxylase, were measured in fetal and adult animals. Furthermore, a series of experiments were conducted using fetal rabbit lung explants to examine the substrate requirements for normal glycogen synthesis during mid-gestation.

Methods

Timed-pregnant New Zealand white rabbits were anesthetized with sodium pentobarbital via the ear vein. Rabbit fetuses were quickly delivered by caesarian section and placed immediately into ice-cold saline. Fetal and adult lungs were cleaned of non-respiratory tissue and made into a 10% homogenate in a medium containing 0.2 M sucrose -10mM Tris-HCl (pH 7.50) -1 mM glutathione-

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1 mM EDTA. The homogenization was carried out on ice with a Potter-Elvejhem homogenizer utilizing a motor-driven teflon pestle. Nuclei and cellular debris were removed by centrifugation at 600 x g for 10 minutes at 4° C. A mitochondria-enriched fraction was obtained by centrifuging the supernatant at 6,000 x g at 4° C. The pellet was washed twice with homogenizing medium and finally centrifuged at 6,000 x g for 10 minutes. A cytosolic fraction was obtained by centrifuging the 6,000 x g supernatant at 100,000 x g for one hour at 4° C in a Beckman L2-65B centrifuge. Lactate dehydrogenase was assayed in the cytosolic fraction (11); pyruvate carboxylase and phosphoenolpyruvate carboxykinase were assayed in both the cytosolic and mitochondrial fractions (12). Mitochondria were sonicated on ice prior to assay with four 10 second bursts with a Bronson sonifier, Model 185, set at level 5. The contribution of blood contamination to the lactate dehydrogenase activity was estimated by the method of Cross, et al. (13). Final lactate dehydrogenase activities were corrected for this contamination (11). All enzyme activities were linear over the time and protein concentration used in these studies.

Organ cultures of fetal lungs were prepared essentially as described by Gross and Wilson (14). Fetal rabbit lungs were removed with sterile technique and placed in ice-cold Waymouth's MB 752/1 medium containing 100 U/ml of penicillin and 100 µg/ml of streptomycin. All Waymouth medium was purchased without glucose so that each experimental medium could be supplemented with the appropriate glucose concentration. All media contained glutamine (2.4 mM) and alanine (150µM). The lungs were freed of non-respiratory tissue and minced into 0.8 mm^3 explants with the aid of a McIlwain tissue chopper. The explants were subsequently placed on either side of a 60 mm dish containing 2.5 ml of medium. The explants were placed directly onto scratched areas of the dish to enhance explant adherence. After being allowed to rest for 90 minutes, the explants were rocked from side to side on a rocker platform set at 3 cycles per minute. This permitted the explants to be alternately exposed to culture medium and atmosphere. Explants were incubated at 37° C in an atmosphere of 95% oxygen/5% CO_2 for 48 hours. Medium was replaced every 12 hours to maintain adequate substrate levels. The 3-aminopicolinic acid used in some of the explant studies was graciously supplied by Dr. Henry Lardy.

Results and Discussion

The data in Table 1 demonstrate that lactate dehydrogenase and phosphoenol-pyruvate carboxykinase activities in the fetal lung are approximately two times

TABLE 1

LACTATE DEHYDROGENASE (LDH), PHOSPHOENOLPYRUVATE CARBOXYKINASE (PEPCK), AND PYRUVATE CARBOXYLASE (PYRC) ACTIVITIES IN FETAL AND ADULT LUNG*

AGE	LDH (U/MG PROTEIN)	PEPCK NMOLES/MG/MIN	PYRC NMOLES/MG/MIN
FETAL LUNG (23D.GESTATION)	1.58 ± 0.05	12.78 ± 2.02	2.40 ± 0.31
ADULT LUNG	0.73 ± 0.04	3.34 ± 1.02	2.86 ± 0.24

* DATA ARE EXPRESSED AS THE MEAN ± S.E.M. OF 3-6 DETERMINATIONS

and four times greater, respectively, than in the adult lung. Pyruvate carboxylase activities, on the other hand, were similar in both fetal and adult lungs. The level of pyruvate carboxylase activity found in the fetal lung, however, is sufficient to account for the amount of glycogen accumulated at this gestational age. Twenty-three day gestation animals were chosen for these experiments because this is the period during gestation with the greatest rate of glycogen synthesis in the fetal rabbit lung. As is the case for adult rabbit liver, pyruvate carboxylase and phosphoenolpyruvate carboxykinase activities in the fetal lung were confined predominantly to the mitochondrial fraction. The enzyme activities reported here support the notion that lactate or 3-carbon metabolites of glucose are potentially important sources for fetal lung glycogen deposition.

In order to further determine if lactate is important for fetal lung glycogen deposition, a series of experiments utilizing fetal lungs from 22 day gestation animals was performed. Twenty-two day gestation animals were used as starting material, since the rate of glycogen deposition in fetal lung is greatest from 22 to 24 days. The results of these experiments are presented in Table 2. When fetal lung explants are incubated for 48 hours in a medium containing only glucose, increasing the glucose concentration from 2.5 mM (the approximate physiological concentration at this gestational age) to 20 mM results in a twofold increase in the glycogen content of the explants. The addition of lactate to medium containing 2.5 mM glucose also produces an increase in measured glycogen content. When the medium contains both 2.5 mM glucose and 10 mM lactate, the amount of glycogen in the fetal lung explants was similar to that found when the medium contained 20 mM glucose alone. The amount of glycogen formed in these explants, even under the best conditions, is approximately 62% of that found in the intact tissue. Thus, the cultured explants are not depositing glycogen at a rate equivalent to the in vivo rate. This may be due to the absence in the culture medium of hormones or mediators necessary for maximum glycogen accumulation. The addition to the media of 150 μg/ml 3-aminopicolinic

TABLE 2

GLYCOGEN CONTENT OF FETAL RABBIT LUNG EXPLANTS*

CULTURE CONDITIONS		GLYCOGEN CONTENT
GLUCOSE MM	LACTATE MM	μMOLES GLUCOSE EQUIVALENTS PER MG PROTEIN
2.5	0	0.48 ± 0.05 (3)
5.0	0	0.56 (2)
10.0	0	0.78 (2)
20.0	0	0.98 (1)
2.5	2.5	0.59 (2)
2.5	5.0	0.86 (2)
2.5	10.0	0.99 ± 0.04 (3)

*DATA ARE THE MEAN OF (N) EXPERIMENTS, EACH DONE IN DUPLICATE.

acid, a known inhibitor of phosphoenolpyruvate carboxykinase (15), substantially reduced glycogen accumulation even in the absence of medium lactate (data not shown). The diminution of glycogen content by 3-aminopicolinic acid in the presence of glucose alone can be explained in the following manner. The fetal lung is known to be a net producer of lactate in the absence of medium lactate. Thus, when glucose is the sole carbohydrate source in the medium, explants would produce significant quantities of lactate, which could subsequently be re-utilized for glycogen synthesis. The presence of 3-aminopicolinic acid would block this reutilization, resulting in diminished overall glycogen content.

In summary, this report indicates that at a time during gestation when the fetal lung is forming significant quantities of glycogen, important enzymes for the utilization of 3-carbon substrates are present and elevated over adult values. The adult lung, in contrast to the fetal organ, does not actively engage in glycogen metabolism and would therefore be expected to have significantly lower enzyme activities. Furthermore, the results from the explant experiments

indicate that glucose alone is a rather poor substrate for fetal lung glycogen accumulation. The combination of lactate and glucose, in approximately physiological concentrations, produces glycogen contents similar to those found when 20 mM glucose alone is used. This scenario is similar to that in the adult liver, where glucose, lactate, and glucogenic amino acids are required to synthesize significant quantities of glycogen (16). Although lactate may be acting to stimulate glucose incorporation into fetal lung glycogen, it is more likely that lactate carbons are being utilized directly for glycogen synthesis. Confirmation of this hypothesis will require direct incorporation studies utilizing in vivo or in vitro systems with radiolabelled precursors.

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