6. Kaliss, N., Cancer Res. **18**, 992 (1958).
7. Morgan, J. F., Guerin, L. F., and Morton, H. J., Cancer Res. **16**, 907 (1956).
8. Mirand, E. A. and Hoffman, J. G., Proc. Soc. Exptl. Biol. Med. **88**, 656 (1955).
9. Koprowski, H., Theis, G., and Love, R., Proc. Roy. Soc. (London), Ser. **166**, 37 (1956).
10. Yaffe, D. and Feldman, M., J. Natl. Cancer Inst. **23**, 133 (1959).
11. Möhler, G. and Möhler, E., J. Clin. Pathol. (Suppl.) **20**, 437 (1967).
12. Molomut, N., Cancer Res. **18**, 906 (1958).

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Uridine Diphosphate Galactose Pyrophosphorylase from Calf Liver*† (32845)

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That nucleoside diphosphate sugars represent a metabolically important class of compounds is now well documented (1). Their importance lies not only in their intermediacy in monosaccharide formation, but also in their role in glycosyl transfer reaction. The pyrophosphorylase catalyzed synthesis of uridine diphosphate glucose (UDP-Glc) from uridine triphosphate (UTP) and glucose-1-phosphate (Glc-1-P) is of primary significance.

$$\text{UTP} + \text{Glc-1-P} \underset{}{\overset{\text{pyrophosphorylase}}{\rightleftharpoons}} \text{UDP-Glc} + \text{PP}_i \quad (1)$$

With crude extracts of mammalian tissues, a number of nucleoside diphosphate sugars (NPD-sugars) have been biosynthetically prepared, many of them novel, from the appropriate nucleoside triphosphate and the sugar-1-phosphate (2,3). These NDP-sugars have been prepared where the nucleoside is adenosine, guanosine, inosine, uridine, cytidine, or thymidine and the sugar is glucose, galactose, mannose, or xylose. From calf liver extracts it has been possible to identify and purify about 500-fold a guanosine diphosphate hexose pyrophosphorylase (4) and crystallize a UDP-Glc pyrophosphorylase (5). Neither enzyme is highly specific for substrate at this stage of purification.

Isselbacher (6) has implicated a UDP-galactose (UDP-gal) pyrophosphorylase from rat and bovine liver in an accessory pathway of galactose (Gal) metabolism as follows:

$$\text{Gal} + \text{ATP} \xrightarrow{\text{kinase}} \text{Gal-1-P} + \text{ADP}, \quad (2)$$

$$\text{Gal-1-P} + \text{UTP} \underset{}{\overset{\text{pyrophosphorylase}}{\rightleftharpoons}} \text{UDP-Gal} + \text{PP}_i, \quad (3)$$

$$\text{UDP-Gal} \underset{}{\overset{\text{epimerase}}{\rightleftharpoons}} \text{UDP-Glc}, \quad (4)$$

$$\text{UDP-Glc} + \text{PP}_i \underset{}{\overset{\text{pyrophosphorylase}}{\rightleftharpoons}} \text{UTP} + \text{Glc-1-P}, \quad (5)$$

Sum of Eqs. (3, 4, and 5) $\quad \text{Gal-1-P} \rightleftharpoons \text{Glc-1-P}$

Since the catalyst for Reaction (5) was obtained in cystalline form (5) and the epimerase for Reaction (4) is widely distributed in nature and well described, it became of interest to attempt to characterize the UDP-Gal pyrophosphorylase. Consequently, it appears that in calf liver all activity for UDP-Gal is inseparably associated with the UDP-Glc pyrophosphorylase. The evidence for the common identity of these enzymes together with some considerations of the specificity are the subject of this report.

Procedures. The chemicals and other materials have been described in detail elsewhere (5,7). For estimating enzyme activity in the direction of pyrophosphorolysis, the formation of Glc-1-P (8) or UTP (9) were measured. The chromatographic procedures for identifying the various substrates have been described (10). The extraction of calf liver and the detailed purification and crystallization procedures for UDP-Glc pyrophosphorylase are reported elsewhere (5).

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TABLE I. Summary of the Purification and Crystallization of Uridine Diphosphate Glucose Pyrophosphorylase.

Fraction	Protein (mg)	Specific activity		Ratio
		UDP-Glc[a]	UDP-Gal[a] × 10^2	UDP-Glc/UDP-Gal
1. Crude	101,200	0.43	0.56	77
2. $(NH_4)_2SO_4$(40–58%)	25,900	1.03	1.6	65
3. $Ca_3(PO_4)_2$Gel	15,200	1.50	2.1	71
4. DEAE-cellulose	2,000	10.8	16	68
5. Crystallization	57.4	223	290	77
6. Recrystallization				
1.	38	189	260	73
2.	10	193	310	62
3.	5.6	235	380	61

[a] A unit of enzyme is that amount which catalyzes the pyrophosphorolysis of 1 μmole of substrate/min at 25°C. Specific activity is the units of enzyme/mg of protein.

Results and Discussion. In the initial extracts of calf liver and throughout purification and recrystallization of the enzyme, the catalytic activity towards UDP-Glc and UDP-Gal remains in constant ratio within experimental error. A summary of this purification is given in Table I. It can be calculated from the table that, since the initial 101 gm of protein in the extract contained 43,000 units of UDP-Glc pyrophosphorylase and since the crystalline protein has a specific activity of 235 units/mg, the extract initially contained approximately 180 mg of enzyme. Thus, almost 0.2% of the extractable protein of calf liver is UDP-Glc pyrophosphorylase. Activity for UDP-Gal remained at about 1.5% that of UDP-Glc throughout the isolation and crystallization procedures. It was not possible by the methods employed to find a fraction in the extracts with a higher relative specific activity for UDP-Gal. Electrophoresis on polyvinyl geon resin (11) failed to separate a fraction with a different ratio of activity towards UDP-Glc and UDP-Gal. The occurrence of some activity towards both substrates in a different ammonium sulfate fraction than the one used for crystallization complicates the results somewhat (5). However, crystals have recently been obtained from this second fraction which have been found to have the same ratio of activity towards the two substrates. Thus the ratio of activity towards UDP-Glc and UDP-Gal was similar in all fractions of calf liver.

Using ^{14}C labeled substrates, the formation of UDP-Gal from UTP and Gal-1-P was demonstrated by chromatographic techniques (10). When added as noncompeting substrates, the recrystallized enzyme has a low but significant activity for other NDP-sugars. The reaction rate is appreciable and varies from 0.1 to 4.0% where the uracil of UDP-Glc is replaced by other pyrimidines and purines, and also when glucose is replaced by other saccharides.

Thus as an isolated laboratory reaction UDP-Gal can be synthesized using the severalfold purified and recrystallized enzyme as a catalyst. Within the liver tissue, catalysis of such a reaction would depend on the relative amounts of other substrates for the same enzyme, UDP-Glc, Glc-1-P and Gal-1-P, etc. Until this can be more carefully evaluated with appropriate studies with human liver, caution should be exercised in attributing significance to the pyrophosphorylase pathway for the metabolism of galactose.

Summary. The biosynthesis of UDP-Gal from UTP and Gal-1-P is catalyzed by extracts of calf liver. At all stages of purification and after several recrystallizations, catalytic activity for UDP-Gal is inseparably connected with the enzyme catalyzing the biosynthesis of UDP-Glc. These findings are discussed from the standpoint of galactose metabolism in the galactosemic.

1. Ginsburg, V., Advan. Enzymol. 26, 35, 1964.
2. Verachtert, H., Bass, S. T., and Hansen, R. G.,

Biochem. Biophys. Res. Commun. 15, 158 (1964).

3. Verachtert, H., Bass, S. T., and Hansen, R. G., Biochem. Biophys. Acta 92, 482 (1964).

4. Verachtert, H., Bass, S. T., Rodriguiz, P., and Hansen, R. G., J. Biol. Chem. 241, 2007 (1966).

5. Albrecht, G. J., Bass, S. T., Seifert, L. L., and Hansen, R. G., J. Biol. Chem. 241, 2968 (1966).

6. Isselbacher, K. J., J. Biol. Chem. 232, 429 (1958).

7. Carlson, D. M. and Hansen, R. G., J. Biol. Chem. 237, 1260 (1962).

8. Munch-Petersen, A., Acta. Chem. Scand. 9, 1523 (1955).

9. Verachtert, H., Bass, S. T., Seifert, L. L., and Hansen, R. G., Anal. Biochem. 13, 259 (1965).

10. Verachtert, H., Bass, S. T., Wilder, J., and Hansen, R. G., Anal. Biochem. 11, 497 (1965).

11. Kunkel, H. G. and Slater, R. J., Proc. Soc. Exptl. Biol. Med. 80, 42 (1952).

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"Alveolar" and Whole Lung Phospholipids of Newborn Lambs* (32846)

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The mammalian lung requires a surface-active material for its alveolar stability (1–4). This material, presumably a lipoprotein complex(2,5,6) containing a highly saturated phosphatidylcholine (7–9) can be isolated from the internal surface of the lung by washing with saline.

The purpose of the present study was to determine the qualitative and quantitative nature of "alveolar" phospholipids and of whole lung tissue phospholipids of newborn lambs following the onset of breathing. Such biochemical data are a prerequisite for an understanding of the synthesis and control of secretion of pulmonary surface-active material during the neonatal period.

Materials and Methods. Fifteen newborn lambs were studied; 12 were delivered spontaneously and three were delivered by cesarean section. Each lamb was sacrificed by injection of 1% Xylocaine intracisternally, the trachea was ligated, the lamb was exsanguinated by transection of the abdominal aorta, and the lungs were removed. The right major bronchi were ligated and the left lung was separated. About 60 ml of normal saline was introduced by a syringe into the left lung through a cannulated trachea. The saline was withdrawn and returned to the lung five times. Care was taken to wash both the upper and lower lobes uniformly. The left lung was then sequentially washed five times, each with 30 ml of saline for a total of 1200 ml. Each 30-ml portion of lung wash was shaken several times and then centrifuged at 1000*g* at 5°C for 10 min to remove cells and debris. The supernatant was lyophilized.

In order to estimate the reliability of lung wash as a sampling procedure, 32 sequential saline washings of 30 ml each were carried out on the left lungs of three lambs. The lipid phosphorus was measured in the lyophilized lung wash of each 60-ml (two 30-ml washes) cell-free fraction.

Lipid analysis. The lipid was extracted from the lyophilizate with $CHCl_3$–MeOH (2:1, v/v) and washed(10). Paired samples of the extract were taken to dryness and analyzed for lipid phosphorus content(11). The lipids were fractionated into neutral lipid, nonacidic and acidic phospholipids on a DEAE[1] cellulose acetate column. The DEAE cellulose was activated according to Rouser *et al.*(12).

The lipids were eluted from the column according to the elution schema described by Gluck *et al.*(13) and the lipid phosphorus was determined on each eluent fraction. To estimate the percentage distribution of each

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[1] Diethylaminoethyl from Applied Science Laboratories.