In all compounds studied, the hydroxy group and the chlorine atom were in the ortho position relative to each other and no further relationship (e.g. meta and para) was investigated. All compounds also gave hydroxylated metabolites formed by either direct hydroxylation or hydroxylation via arene oxide intermediates. Compound **4a** was further dechlorinated to phenol (**4b**). From compound **3** 3 more metabolites formed via reductive dechlorination (compounds **3e**, **3f** and **3g**, see scheme) were isolated. By studying the specific fragmentation pattern, the relative intensities and the retention times and by comparison with data of similar compounds from earlier studies[9,10,13], the structures of the compounds shown in the Scheme could be determined. In all cases final proof was obtained by synthesis. Because the compounds **3b**, **3c** and **3d** are major metabolites, whilst **3a**, **3e**, **3f** and **3g** represent only a few percent of the total amount of metabolites formed, most likely **3e**, **3f** and **3g** were formed via reductive dechlorination of **3b**, **3c** and **3d** respectively, since the latter all have a hydroxy group ortho to a chlorine atom. The present data however, do not rule out formation of **3e**, **3f** and **3g** via the 3′, 4′-epoxide of **3a**, similar to 3′, 4′-epoxidation of **3**. The relative amounts of the unchanged compounds, their hydroxylated and dechlorinated metabolites isolated from the urine are given in the table.

Biosynthesis of phosphatidylethanolamine from CDP-ethanolamine by the Golgi complex of rat liver in vitro[1]

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Summary. A Golgi-rich fraction from rat liver has been shown to synthesize phosphatidylethanolamine from CDP-ethanolamine in vitro. The implications of the existence of such a pathway for the membrane flow hypothesis are discussed.

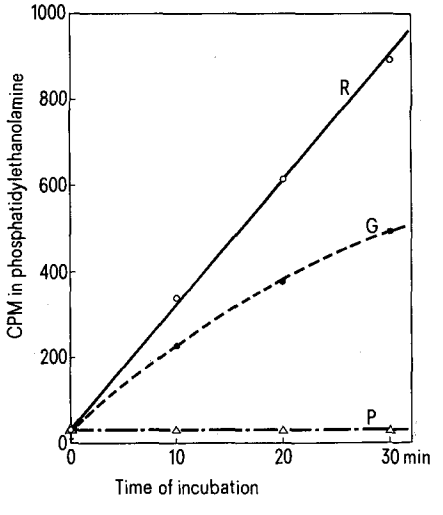
The biosynthesis of phospholipids has been shown to occur in endoplasmic reticulum[4–6] and in mitochondria[7], whereas the Golgi complex has been reported to lack some of the enzymes required in such biosynthesis[5,6]. However, this report presents evidence that the Golgi-rich fractions from rat liver can synthesize phosphatidylethanolamine from CDP-ethanolamine in vitro, demonstrating that at least the terminal enzyme is present. The rough endoplasmic reticulum showed similar synthetic activity while the plasma membrane showed no incorporation of CDP-ethanolamine.

The reaction under study was as follows:

1,2-diacyl-sn-glycerol + CDP-ethanolamine*
ethanolamine phosphotransferase
→ phosphatidylethanolamine* + CMP

When CDP-ethanolamine was radioactively labelled with ^{14}C in the ethanolamine moiety, the recovery of radioactive phosphatidylethanolamine measured the activity of ethanolamine phosphotransferase, an enzyme responsible for the final step of phosphatidylethanolamine de novo synthesis according to the Kennedy pathway.

Materials and methods. All subcellular fractions were prepared from the livers of male Wistar rats weighing 200–230 g. The rough microsomal fractions were prepared by Dallner's method[8] and Golgi-rich fractions by the method of Sturgess, et al.[9]. The plasma membrane frac-



Recovery of radioactive phosphatidylethanolamine from CDP-ethanolamine vs time of incubation with rough microsomal, Golgi-rich and plasma membrane fractions of the rat liver.
Rough microsomal (R, 232 μg of protein), Golgi-rich (G, 139 μg of protein) or plasma membrane (P, 117 μg of protein) fractions were each incubated at 37 °C in a medium containing CDP-[^{14}C]-ethanolamine and diglyceride (see Materials and methods) in quadruplicate. At each time interval: 0, 10, 20 and 30 min, incubation was terminated, phospholipids were extracted from the total incubation mixture and separated by TLC. The spot corresponding to phosphatidylethanolamine (PE) was scraped and counted in a liquid scintillation system.

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tion, prepared according to Ray's procedure[10], was kindly supplied by Dr J. R. Riordan. Using quantitative morphometric techniques in the electron microscope, the composition of these fractions has been characterized as 82% of rough microsomes, at least 70% of Golgi complexes and 88% of plasma membranes in the 3 fractions respectively.

The subcellular fractions were frozen and dispersed in distilled water by sonication just before incubation. 1,2-diacyl-sn-glycerol dissolved in hexane was purchased from Serdary Research Laboratory, London, Ontario. An aliquot was dried under nitrogen and dispersed by sonication for 2 min in 0.1 M Tris buffer, pH 7.4, containing 0.03% tween 20. Cytidine 5'-diphospho[2-^{14}C]-ethan-1-ol-2-amine (Amersham Searle) was diluted with distilled water to contain 1 μCi/ml at a specific radioactivity of 28 mCi/mmole. The incubation medium, based on that used by Van Golde, et al.[5], consisted of the following: 50 μl of 1,2-diacyl-sn-glycerol (5 mg/ml), 10 μl of CDP-ethanolamine (2 mM), 20 μl of cytidine 5'-diphospho(2-^{14}C)ethan-1-ol-2-amine (1880 cpm/μl), 10 μl of glutathione (0.1 M), 25 μl of $MgCl_2$ (0.1 M), 85 μl of Tris (0.1 M, pH 7.4 in 0.03% tween 20) and 50 μl of subcellular fraction (2–5 mg of protein/ml). Samples were incubated at 37 °C for 0, 10, 20 and 30 min and then 0.94 ml of chloroform:methanol (1:2 v/v) was added. Total phospholipids were extracted according to the method of Bligh and Dyer[11] and separated on TLC. The spot corresponding to phosphatidylethanolamine was scraped and counted in Aquasol (New England Nuclear). Protein was determined according to the method of Lowry, et al.[12], with bovine serum albumin as standard.

Results and discussion. The figure illustrates the recovery of radioactivity in phosphatidylethanolamine after 0 to 30 min of incubation at 37 °C in rough microsomal, Golgi-rich and plasma membrane fractions. The rough microsomal fraction (R) incorporated radioactive CDP-ethanolamine into phosphatidylethanolamine rapidly at a rate proportional to the time of incubation. The Golgi-rich fraction (G) incorporated radioactivity into phosphatidylethanolamine rapidly but the rate of incorporation decreased with time. The plasma membrane fraction (P) showed no significant incorporation of radioactivity.

The specific activity of ethanolamine phosphotransferase was measured as nmoles of CDP-ethanolamine transferred/min mg of protein at 37 °C. The specific activity in the rough microsomal fraction was 0.070 nmoles/min mg of protein and that in the Golgi-rich fraction was 0.069 nmoles/min mg of protein. The data show that the Golgi complex of the rat liver actively synthesized phosphatidylethanolamine. This was not due to activity of contaminating microsomes because the Golgi fractions contained at least 70% of Golgi membranes with the major contaminant being plasma membrane and with only minimal contamination by rough microsomes.

Evidence from studies in vivo has supported our findings that at least some phosphatidylethanolamine was synthesized de novo and incorporated into the membranes of the Golgi complex (Chang, et al., manuscript submitted for publication). Thus, if the Golgi complex actively generates its own membrane phosphatidylethanolamine by de novo synthesis, its role in membrane biogenesis may not be restricted solely to membrane differentiation as proposed in the endomembrane flow hypothesis[13]. The specific function(s) of the phosphatidylethanolamine that is independently synthesized in the Golgi complex is being further examined.

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Angiotensin I converting enzyme activity in pulmonary tissue of fetal and newborn rabbits[1]

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Summary. Angiotensin I converting enzyme in pulmonary tissue of fetal and newborn rabbits was measured using Hip-His-Leu as substrate. Enzyme activity was detected in the late fetal period, increased gradually until birth and increased markedly after birth. Enzyme activity reached adult levels on the 2nd and 3rd day after birth. This observations suggests that the metabolic activity of the lung for angiotensin develops suddenly at the time of derivery.

The non-respiratory function of the lung have been investigated recently[2–5]. Many vasoactive substances are metabolized in the pulmonary circulation. These functions are considered to have an important role for regulation on the level of these substances in the systemic circulation[6]. It is interesting to know when the metabolic function of the lung for vasoactive substances does develop during fetal life.

In the present study, angiotensin I converting enzyme (carboxydipeptidase) activity in pulmonary tissue was measured during the fetal and neonatal life in rabbits. This enzyme transforms angiotensin I to angiotensin II and inactivate bradykinin by releasing a dipeptide from the C-terminal of the peptides.

Materials and methods. Male and female rabbits were kept in the same cage overnight and the first day of the gestation was calculated. Fetuses were excised from uterus under light anesthesia with pentobarbital sodium. The

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