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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lungs were removed and washed with chilled saline solution, then weighed and kept in −20 °C until use. All materials were measured within 3 days after freezing. The enzyme assay was performed spectrophotometrically according to Cushman and Cheung[7]. Synthetic Hip-His-Leu (obtained from the Institute for Protein Research, Osaka University, Osaka, Japan) was used as the substrate, and formed hippuric acid was measured by absorbancy at 228 nm. The activity was expressed as μmole/g of lung/min.

For the preparation of the enzyme material, the lung was homogenized with Polytron® (Luzern, Switzerland) for 60 sec at power control 6 with 10 times volume of 10 mM of phosphate buffer pH 8.3 for fetal lung, and with 200 times volume for adult lung, then centrifuged at 1500 rpm for 10 min. The supernatant was used as the enzyme material.

The development of b. wt, lung weight and angiotensin I converting enzyme in fetal neonatal rabbit

	Body weight (g)	Lung weight (mg)	ATI-CE activity (μmole/g/min)[a]
Gestational days			
16(n = 6)	2.9 ± 0.02[b]	55.8 ± 0.9	0
24(n = 7)	13.9 ± 0.98	390.6 ± 26.9	0.30 ± 0.02
30(n = 8)	40.7 ± 1.2	1210 ± 50	0.89 ± 0.07
31(n = 9)	47.6 ± 1.7	1130 ± 50	1.54 ± 0.59
Days after birth			
1[c](n = 4)	30.8 ± 3.09	662.0 ± 86.0	1.88 ± 0.19
2(n = 13)	50.6 ± 0.65	843.0 ± 63.6	2.98 ± 0.21
3(n = 10)	46.5 ± 0.71	855.6 ± 29.7	3.40 ± 0.23
10(n = 2)	74.5[d]	1125	3.75
Adult (n = 10)			3.7 ± 0.1

[a] ATI-CE: Angiotensin I-converting enzyme; [b] mean ± SE; [c] 6 h after birth; [d] average.

Results and discussion. As shown in the table, b.wt and lung weight increased gradually until birth, followed by a decrease for few h. On the 2nd day after birth, the b.wt and lung weight increased again.

Angiotensin I converting enzyme activity was not detected on the 16th day of gestation, detected very low at 24th day of gestation, 1 week before derivery. The enzyme activity increased gradually up to the 30th and 31st day of the gestation and increased suddenly after birth. On the 1st day of gestation, the lung weight is reduced by almost 50%, probably mostly by dehydration. Consequently, total converting enzyme activity per lung is decreased once after birth. Then the activity increased to the adult level within 2 or 3 days after birth. These results suggest that the metabolic activity of the lung for vasoactive peptides is not essential during fetal life, and becomes important only after birth. Friedli[8] examined the metabolism of bradykinin in the pulmonary vasucular bed of the fetal and newborn lamb, and showed about half of the capacity to inactivate bradykinin in the fetal lamb at term compared with the mature ewes. In the preterm fetus, no inactivation could be demonstrated in the pulmonary bed.

The pulmonary surfactant content of the lung was reported to show significant increase on the 21st and 22nd day of gestational days in rats[9]. The developmental behaviour of the surfactant was the same as was found in our experiment in rabbits concerning angiotensin I converting enzyme. Pulmonary surfactant is thought to have some relationship to the maturation of the lung, angiotensin I converting enzyme might also have a close relationship to the maturation of the lung. The mechanism of sudden increase of the enzyme activity is under investigation.

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Occurrence of Asn[2], Leu[5]-caerulein in the skin of the African frog Hylambates maculatus

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Summary. The skin of the African frog, Hylambates maculatus, contains large amounts of a caerulein-like peptide, which has been identified as Asn[2], Leu[5]-caerulein. The cholecystokinetic activity of Hylambates-caerulein is very similar to that of caerulein.

A new caerulein-like peptide has been isolated from methanol extracts of the skin of Hylambates maculatus, an African frog belonging to the Ranidae family (subfamily Hyperolineae).

The amino acid composition and sequence of Hylanbates-caerulein differs from that of caerulein only in that the 2 aminoacids, glutamine[2] and theonine[6], of the caerulein molecule are replaced by asparagine and leucine, respectively[2].

Pyr-Gln-Asp-Tyr(SO_3H)-Thr-Gly-Trp-Met-Asp-Phe-NH_2
Caerulein

Pyr-Asn-Asp-Tyr(SO_3H)-Leu-Gly-Trp-Met-Asp-Phe-NH_2
Hylambates-caerulein

Thus, Hylambates-caerulein may be considered as Asn[2], Leu[5]-caerulein.

Materials. The fresh skins of 92 specimens of Hylambates maculatus collected at St. Lucia, Zululand (South Africa), during the period 1973–1975 were used this study. The material weighed 14 8g (average 1.6 g per fresh skin). The skins were removed from the frogs immediately after killing and extracted twice with a volume of methanol 5

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