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

1 Supported in part by grants from the Consiglio Nazionale delle Ricerche, Roma, Italy.
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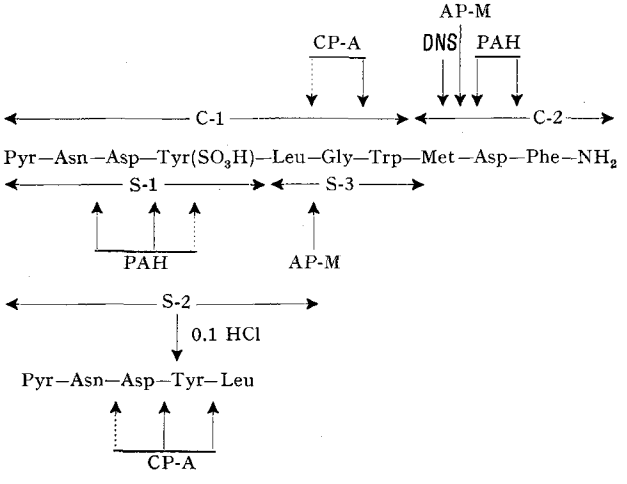
times the weight of the tissue. The extracts were combined and filtered.

Isolation procedure. An aliquot of extract corresponding to 100 g of fresh skin was evaporated to dryness, the residue washed with petroleum ether and then taken up in water plus 99% ethanol to give a final ethanol concentration of 95%. The liquid was passed through 2 columns of alkaline alumina Merck 90 (activity grade 1), each of 170 g, which were then eluted with ethanol-water mixtures of decreasing concentrations of ethanol, each of 200 ml. The peak of caerulein-like activity (guinea-pig gall bladder) emerged in the 2 50% ethanol eluates, which contained altogether approximately 35 mg of peptide, expressed as caerulein, and 50% of the activity put on the column.

The suspicion that Hylambates-caerulein might be different from caerulein was aroused by the fact, that in comparison to caerulein, Hylambates-caerulein was eluted by higher concentrations of ethanol and with considerably better yields.



Hylambates-caerulein present in the 50% ethanol eluates was further purified by preparative electrophoresis. The single biologically active peptide spot was positive to chlorine, to the Ehrlich reagent for tryptophan, to the α-nitroso-β-naphthol reagent for tyrosine, to the jodoplatinate reagent for sulphur aminoacids, but it was negative to ninhydrin. On ascending thin layer chromatography on silica gel, Hylambates-caerulein had an R_f 0.35 in the solvent system n-butanol-acetic acid-water (4:1:1), and on high voltage electrophoresis the peptide spot migrated toward the anode at neutral and acid pHs, its position being 0.5 relative to glutamic acid at pH 5.8 and 0.55 relative to cysteic acid at pH 1.9.

Structure. The structure of Hylambates-caerulein was deduced by sequential analysis of the fragments obtained by digestion with chymotrypsin(C) and subtilisin (S) followed, as shown in the chart, by digestion with carboxypeptidase A (CP-A), aminopeptidase M (AP-M), partial acid hydrolysis (PAH) and dansylation (DNS). The dipeptide Pyr-AsnOH was identified by its electrophoretic behaviour.

It is possible that the small amounts of caerulein-like activity (guinea-pig gall bladder) found in extracts of Kassina senegalensis (1–5 μg/g fresh skin) and of Phlyctimantis verrucosus (5–7 μg/g dry skin) are due, at least in great part, to Asn[2], Leu[5]-caerulein.

Hylambates-caerulein displayed on the isolated and in situ guinea-pig gall bladder, as well as on the isolated guinea-pig ileum and rabbit large intestine, stimulant effects which were qualitatively identical with those elicited by caerulein. From a quantitative point of view, definitive conclusions are not possible because pure synthetic Asn[2], Leu[5]-caerulein was not available. However, the 2 peptides may be considered approximately equiactive.

In addition to Asn[2], Leu[5]-caerulein, methanol extracts of the skin of Hylambates maculatus contain 4 or 5 other active peptides, mainly belonging to the tachykinin family. The isolation of 2 of them is in progress.

Biliary excretion and metabolism of ^{14}C cimetidine following oral administration to male and female rats

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Summary. In rats, the bile is not a major route of excretion of cimetidine or its metabolites, since only 10% of the ^{14}C associated with an oral dose of labelled cimetidine was excreted in the bile during 8 h after dosing.

Cimetidine is an orally active histamine H_2-receptor antagonist[1], marketed under the trade mark 'Tagamet'. When this compound was given orally to male rats[2,3] (30 mg/kg of cimetidine labelled with ^{14}C in the 2-position of the imidazole ring; figure) 58% of the radioactivity was excreted in the urine during the 24 h after dosing and approximately 50% of this was unchanged cimetidine. Similarly, 64% of the radioactivity administered orally to female rats was excreted in the urine within 24 h, but a larger proportion of the eliminated ^{14}C was associated with unchanged cimetidine. The significance in rats of biliary excretion of cimetidine and its metabolites has not been reported previously, and was the subject of the separate study described in this communication.

Materials and methods. 4 male and 4 female Wistar rats, weighing 198–201 g and 194–203 g respectively, each received by gastric intubation, 30 mg/kg of 2-^{14}C-cimetidine dissolved in 0.9% (w/w) saline. This resulted in the administration of 10 μCi of ^{14}C to each animal. After dosing, the animals were anaesthetized with 2% halothane in oxygen containing 5% CO_2, and the bile duct cannulated

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