Physalaemin may be distinguished in parallel assays from all other known polypeptides, including eledoisin[2]. However, eledoisin is the polypeptide to which physalaemin seems so far to be most similar in its pharmacological properties.

Like eledoisin, physalaemin potently stimulates movements and tonus of the isolated rabbit large intestine (threshold concentration: extract corresponding to 1–2 μg fresh tissue per ml nutrient liquid), the isolated guinea-pig ileum (2–6 μg fresh tissue/ml), and other preparations of gastro-intestinal smooth muscle as well (rat stomach, rat duodenum, frog stomach, etc.); like eledoisin, physalaemin is not very active on the oestrus uterus of the rat.

The blood pressure of the anaesthetized dog and rabbit is potently lowered by the intravenous injection of *Physalaemus* extracts. Intensity and duration of hypotension are proportional to the injected dose of physalaemin and there is no sign of tachyphylaxis. A short-lived but evident fall of blood pressure may be produced in the dog by the intravenous injection of the extract corresponding to 5 μg fresh skin per kg body weight.

The accompanying Table gives some approximate activity equivalencies for the extract corresponding to 1 g fresh *Physalaemus* skin.

	Approximate equivalencies for 1 g fresh *Physalaemus* skin			
	Eledoisin	Substance P	Bradykinin	Histamin
Dog blood pressure	300–400 μg	20000 to 30000 μ	>20 mg	> 20 mg
Rabbit large intestine	150–200 μg	50000 μ	>15 mg	>100 mg
Rat uterus	30–50 μg	50–200 μ	< 1 μg	inhibition
Rat duodenum	100 μg	–	inhibition	–
Guinea-pig ileum	100–200 μg	–	0.5–2 mg	–

Rabbit large intestine, guinea-pig ileum and dog or rabbit blood pressure are particularly suitable for the quantitative bioassay of physalaemin, owing to their high sensitivity and the excellent dose/response relationship.

Crude *Physalaemus* extracts do not apparently contain other active substances with the possible exception of small amounts of a bradykinin-like polypeptide. A biologically pure physalaemin preparation may be obtained by absorption of the crude *Physalaemus* material dissolved in 95% ethanol on an alkaline alumina column and subsequent elution with descending concentrations of ethanol, followed by ion-exchange chromatography on a column of Amberlite CG-50.

The occurrence of physalaemin is now being investigated in other *Physalaemus* species, as well as in numerous other amphibians gathered throughout the world.

A full report on the pharmacological properties of physalaemin will be published elsewhere. The isolation of the polypeptide and the elucidation of its structure is in progress.

Riassunto. Gli estratti di pelle fresca o secca di *Physalaemus fuscumaculatus* contengono una sostanza attiva di natura polipeptidica, la *physalaemina*, dotata di potente azione ipotensiva e di intensa azione stimolante su alcuni muscoli lisci extravasali. La physalaemina è facilmente distinguibile, mediante saggi paralleli, da tutti gli altri polipeptidi biogeni finora noti, compresa la eledoisina che alla physalaemina s'accosta per parecchie delle sue azioni farmacologiche.

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Istituto di Farmacologia, Università di Parma (Italy), and Instituto de Biologia, Universidad Nacional de Cuyo, Mendoza (Argentine), July 30, 1962.

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Occurrence of Bradykinin-Like Substances in the Amphibian Skin[1]

Besides being an enormous store-house of indolealkylamines, phenylalkylamines and imidazolealkylamines, the amphibian skin appears to be an important production and/or storage site of highly active polypeptides.

One of them, physalaemin, has been described in a preceding communication[2]. In this preliminary report, the occurrence of bradykinin-like substances will be briefly described. The term bradykinin-like should be interpreted in a broad sense.

So far about eighty amphibian species, collected in all parts of the world, but especially in South America, have been studied. High, and sometimes enormous, amounts of bradykinin-like compounds appeared to be present in methanol extracts of the skin of the examined *Phyllomedusae* (*Phyl. sauvagi, Phyl. rhodei*) and *Ranae* (*R. esculenta, R. temporaria, R. pipiens, R. warschewitschii, R. japonica, R. catesbiana, R. nigromaculata*).

The polypeptide nature of the active skin constituents is shown by their rapid and complete inactivation produced by chymotrypsin incubation, and by some preliminary results obtained following acid hydrolysis of the eluates of active paper chromatographic spots.

The accompanying Table presents the activity, expressed in terms of pure bradykinin, of crude methanol extracts of the *Phyllomedusa* and *Rana* species examined. It should be emphasized that the figures are merely indicative of the relative potency of action possessed by the crude skin extracts on the different test-objects. Bradykinin served only as a standard substance, and very often the response to skin extracts was even qualitatively different from that given to bradykinin. In reality, all or nearly all aspects of the biological activity of crude *Phyllomedusa* or *Rana* extracts are due to a more or less complex mixture of active polypeptides. To elucidate the composition of this mixture, each extract should be studied separately and singularly.

[1] Supported by grants from the Consiglio Nazionale delle Ricerche, Roma, and the Rockefeller Foundation, New York.

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Activity of crude extracts of amphibian skin expressed as bradykinin (μg per g tissue)

		Dog blood pressure	Rat uterus	Guinea-pig ileum	Dog large intestine	Rabbit large intestine
Phyllomedusa sauvagi 1962	(dry skin)	800	40–90	75–200	150–200	
Phyllomedusa sauvagi 1962	(wet skin)	1200	20–50	50–150	50–100	
Phyllomedusa rhodei 1962	(dry skin)	1500–2000	250–350	500–650	300–450	
Rana esculenta 1961	(wet skin)	—	7–20	15–20	15–30	300–400
Rana temporaria 1961	(wet skin)	—	90–100	100–130	100–130	—
Rana pipiens 1962	(dry skin)	40	5–10	100	—	—
Rana warschewitschii 1962	(dry skin)	60	70	50	—	>100
Rana japonica 1962	(dry skin)	—	10	<1.5	—	>100
Rana catesbiana 1962	(dry skin)	—	2.5	<1.5	—	>100
Rana nigromaculata 1962	(dry skin)	—	2	1.6	—	>100

So far, a partial separation of the active constituents of crude skin extracts has been carried out for *Phyllomedusa sauvagi* and *Phyllomedusa rhodei*.

In a typical experiment, the residue left by evaporation of the methanol extract of 6 g fresh skin of *Phyllomedusa sauvagi* was dissolved in 95% ethanol and absorbed on an alkaline alumina column. Elution was carried out with descending concentrations of ethanol. At least three peaks of activity appeared in the eluates: the first in 70% ethanol (polypeptide A), the second in 60% ethanol (polypeptide B), and the third in 50–40% ethanol (polypeptide C).

Polypeptide A was the one that most strictly resembled bradykinin in its pharmacological properties. However, its identity with bradykinin is improbable.

Polypeptide B, perhaps the most interesting of the series, possessed a powerful hypotensive action in the dog and the rabbit. The fall of blood pressure which could be obtained with very low doses of the polypeptide, lasted considerably longer than that caused by bradykinin, substance P, eledoisin or physalaemin doses which produced a pressure fall of the same intensity.

Polypeptide C was particularly active on the guinea-pig ileum but the shape of the contraction curve differed sharply from that produced by bradykinin.

Polypeptides identical or similar to polypeptide A have been found in *Phyllomedusa rhodei*, *Rana esculenta* and *Rana temporaria*.

Research is in progress, intended to separate the different bradykinin-like polypeptides which have already been identified and which will be identified in the skin of the new amphibian species which we are collecting throughout the world, to determine their origin and significance, to subject the different polypeptides to a thorough pharmacological study, and finally to isolate the most abundant of them in a pure form in order to elucidate their chemical structure[3].

Riassunto. Gli estrati di pelle di alcuni comuni anfibi nostrani (*Rana esculenta, Rana temporaria*) e, ancor più, di certi anfibi sudamericani del genere *Phyllomedusa* contengono rilevanti quantitativi di polipeptidi bradichinino-simili. Negli estratti di *Phyllomedusa sauvagi* ne sono stati identificati almeno tre, a mezzo di una semplice cromatografia su colonna di allumina basica: polipeptide A, assai simile alla bradichinina per le sue proprietà farmacologiche; polipeptide B, dotato di prolungata ed intensa azione ipotensiva; polipeptide C, particolarmente attivo sull'ileo di cavia. Sono in corso ricerche dirette all'isolamento di questi polipeptidi e al chiarimento della loro struttura chimica.

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[3] We are indebted to, and wish to thank Dr. N. URAKAWA (Department of Veterinary Pharmacology, Faculty of Agriculture, University of Tokyo) for the specimens of *Rana japonica, Rana catesbiana* and *Rana nigromaculata* as well as for specimens of other Japanese amphibian species. A complete study of these species will be published in collaboration with Dr. URAKAWA.

Alkaloide aus *Vinca minor* L.[1] Vincadin, Minovin und Vincorin

Aus *Vinca minor* L. wurden bisher 8 Alkaloide isoliert und beschrieben: Vincamin (Minorin)[2–5], Vincin[6], Vincaminin (Vincarein)[7,8], Vincinin[7], Vincanorin[8], Vincaminorin[9], Vincaminorein[10] und Vincamidin[10]. Drei Alkaloide, Isovincamin[3,6], Perivincin[6,11] und Vincamirin[12] wurden

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