relative spezifische Aktivität nahm unter Theophyllineinwirkung in den ersten 10 min bis etwa zum 3fachen der Kontrollwerte zu. Die endgültige Markierung des intrazellulären Ca (Werte nach 30 und 60 min) wurde durch Theophyllin jedoch nicht beeinflusst. – Die ^{45}Ca-Abgabe wurde durch Theophyllin gegenüber den Kontrollen ebenfalls beschleunigt. – An ruhenden Vorhöfen beeinflusste Theophyllin die ^{45}Ca-Aufnahme und -Abgabe nicht.

Amplitude und Anstiegssteilheit der schnellen Depolarisationsstufe wurden durch Theophyllin verstärkt (Figur 2). Bei dem abgebildeten Versuch hat die Anstiegssteilheit 5 min nach Zugabe von 10^{-3} g/ml Theophyllin von 1,05 auf 2,22 V/sec und die Amplitude von 65 auf 75 mV zugenommen. Gleichzeitig kam es nach Theophyllin zu einer Zunahme der Kontraktionskraft. Die Veränderungen der schnellen Depolarisationsstufe und der Kontraktion nach Theophyllinzugabe bildeten sich mit der gleichen Geschwindigkeit aus. Beide Messgrössen wurden auch bei Änderung der Theophyllinkonzentration im Bereich zwischen 10^{-4} und 10^{-3} g/ml gleichsinnig verändert. Die Schwelle für die schnelle Depolarisationsphase und für die Kontraktion (Figur 2, gestrichelte Linie) sowie das Ruhepotential wurden durch Theophyllin nicht beeinflusst.

Diskussion. Die Wirkung von Theophyllin auf den ^{45}Ca-Umsatz besteht in einer Beschleunigung der ^{45}Ca-Aufnahme und -Abgabe bei unveränderter Markierbarkeit des zellulären Ca. Die elektrophysiologischen Versuche ergaben eine gleichsinnige und gleichzeitige Beeinflussung von elektrischen und mechanischen Messgrössen durch Theophyllin. Insgesamt gesehen ist es nach diesen Befunden wahrscheinlich, dass Theophyllin die Membranpermeabilität für Ca-Ionen während der Erregung steigert. Dadurch könnte es unter dem Einfluss von Theophyllin während des Aktionspotentials zu einem gesteigerten Einstrom von Ca-Ionen aus dem Extrazellulärraum und damit vorübergehend zu einer Zunahme der intrazellulären Konzentration an freien Ca^{++} kommen, die die Zunahme der Kontraktionskraft bewirken würde. Hinweise dafür, dass Theophyllin in ausschliesslich positiv inotrop und nicht toxisch wirkenden Konzentrationen etwa wie Herzglykoside die austauschbare Ca-Fraktion erhöhen[9,10], zum Beispiel durch einen Einfluss auf das sarkoplasmatische Retikulum und andere intrazelluläre Bindungsstellen[11], wurden nicht beobachtet. Vielmehr scheint die Wirkung von Theophyllin am Warmblüterherzen qualitativ derjenigen von Adrenalin zu entsprechen, das ebenfalls den ^{45}Ca-Umsatz bei Meerschweinchenvorhöfen beschleunigt[12] und den elektrophysiologisch messbaren Ca-Einstrom während der Erregung an verschiedenen Herzpräparaten steigert[3,13–15].

Summary. In isolated, electrically driven, left guinea-pig atria, theophylline (5×10^{-4} g/ml) increased the rate of ^{45}Ca uptake and release without affecting the total myocardial Ca content and the amount of exchangeable cellular Ca. In sheep and calf heart preparations, theophylline (10^{-4}–10^{-3} g/ml) increased Ca inward current during excitation (as examined indirectly by Ca dependent changes of membrane potential in TTX-containing solutions) as well as tension development. It is concluded that the positive inotropic effect of theophylline in mammalian hearts is due to an increase in Ca influx during the excitation process.

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Occurrence of Phyllomedusin, a Physalaemin-Like Decapeptide, in the Skin of *Phyllomedusa bicolor*[1]

Methanol extracts of the skin of the South American amphibian *Phyllomedusa bicolor* contain a decapeptide possessing a biological activity very similar to that of physalaemin and eledoisin and a structure closely related to both of them. The isolation and the elucidation of the structure of this peptide are briefly reported.

Eight specimens of *Phyllomedusa bicolor*, captured near Obidos, Parà, Brazil (January 1967), yielded 92 g of fresh skin, which was immediately extracted with methanol. The extract corresponding to 60 g of fresh skin was evaporated to dryness and the residue dissolved in 95% ethanol. The liquid was passed through a column of alkaline alumina which was then eluted with ethanol-water mixtures of decreasing ethanol concentrations. The physalaemin-like activity appeared in the 80% ethanol eluate and the active fraction was found to be almost free of contaminants by chromatographic and electrophoretic criteria. On high voltage electrophoresis on paper, the active spot was found to possess about the same mobility as physalaemin and eledoisin in acidic media ($E_{1.2} = 0.45$ Glu) but, unlike the above peptides, showed a moderately basic character at neutral pH ($E_{5.8} = 0.3$ His). The spot was positive to chlorine but could not be revealed either by ninhydrin, denoting the absence of lysine and of a free terminal amino group, or by the α-nitroso-β-naphthol and Pauly reagents, denoting the absence of tyrosine. Instead, it was positive to the Sakaguchi reagent for arginine.

On total acid hydrolysis, the spot eluted from the electropherograms yielded 1 mole of methionine, leucine, glycine, isoleucine, phenylalanine, arginine, proline and glutamic acid and 2 moles of aspartic acid. Since the amino acid pattern of the 80% ethanol eluate from the alumina column was essentially the same, a further purification was thought to be unnecessary and the eluate was used for the sequential analysis. This consisted, as in the

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case of eledoisin-physalaemin and other peptides[2-4], in the analysis of the fragments obtained by enzymatic degradation of phyllomedusin.

Upon digestion with chymotrypsin it was soon evident that the C-terminal half of the molecule had the same composition and sequence as that of eledoisin.

Pyr-Asn-Pro-Asn-Arg-Phe-Ile-Gly-Leu-Met-NH_2
|— Chym-1 —| |Chym-2| |–Chym-3 | |Chym-4|

Three peptide bonds were, in fact, hydrolyzed with the release of 4 fragments, 2 of which were readily identified as methionine-amide (Chym-4) and as the tripeptide H-Ile-Gly-Leu-OH (Chym-3). Furthermore, the specificity of the chymotryptic attack allowed the precise localization of the phenylalanine residue adjacent to that of isoleucine, as in the C-terminal pentapeptide of eledoisin: -Phe-Ile-Gly-Leu-Met-NH_2. The chymotryptic fragment Chym-2 migrated electrophoretically as a distinctly basic spot ($E_{1.2} = 1.5$ Glu, and $E_{5.8} = 0.84$ His). It was positive to the Sakaguchi reagent and on total acid hydrolysis contained only the arginine and phenylalanine residues. To this fragment the dipeptide structure H-Arg-Phe-OH could be confidently assigned. The last spot (Chym-1) was positive only to chlorine, indicating the lack of free amino groups, it was steady at pH 1.2 and migrated anodically at neutral pH ($E_{5.8} = 0.45$ Glu) indicating an acidic character. Its acid hydrolysate contained 1 mole of glutamic acid, one mole of proline and 2 moles of aspartic acid. Hence, it was clearly the N-terminal tetrapeptide with the composition Pyr-[Pro, 2 Asp]-OH.

Upon tryptic digestion of phyllomedusin only the Arg-Phe bond was hydrolyzed, producing 2 pentapeptides Tryp-1 and Tryp-2.

Pyr-Asn-Pro-Asn-Arg-Phe-Ile-Gly-Leu-Met-NH_2
|—— Tryp-1 ——| |—— Tryp-2 ——|

One of the 2 pentapeptides was the N-terminal half-chain bearing the arginine residue in its C-terminal position, as deduced by the specificity of trypsin. The position of arginine was also indicated by the analysis of Chym-2 and was definitely confirmed by digesting Tryp-1 with carboxypeptidase B which readily liberated this amino acid from the fragment.

The N-terminal tetrapeptide (Chym-1) resulting from the chymotryptic digestion of phyllomedusin, or from digestion of Tryp-1 with carboxypeptidase B, was, on the other hand, completely resistant to the attack of the carboxypeptidases A and B and its sequence had to be deduced by other means.

The electrophoretic migration of phyllomedusin itself (basic in both neutral and acidic media) and of the peptide Tryp-1 (basic at pH 1.2 and neutral at pH 5.8) demonstrated that both the aspartic acid residues were present in the amide form. Furthermore, the chymotryptic attack between Chym-1 and Chym-2 indicated that one of the asparagines had to be bound to the amino group of arginine. This was confirmed by submitting Chym-1 to hydrazinolysis which produced the β-hydrazide of the aspartic acid. The uncertainty of the N-terminal part of the chain, thus restricted to the relative positions of one of the asparagines and of the proline residue, was clorified by partial acid hydrolysis of Chym-1. In the acid hydrolysates obtained by heating the fragment with 0.1 *N* HCl at 100 °C for 1 or 2 h, besides the individual free amino acids, the 2 dipeptides Pyr-Asp-OH and H-Pro-Asp-OH were in fact clearly identified.

Riassunto. Viene descritto l'isolamento e il chiarimento della struttura della phyllomedusina, decapeptide attivo della pelle dell'anfibio sudamericano *Phyllomedusa bicolor*. La phyllomedusina è apparsa strutturalmente assai vicina alla eledoisina e alla fisalemina.

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Cardiotonic Action of 3-Deoxydigitoxigenin

Extending our serial studies on the structure-activity relationship of cardiotonic steroids[1-3], the cardiac action of 3-deoxydigitoxigenin was studied in comparison with that of digitoxigenin.

Experiments were performed on the isolated frog's heart (Straub's preparation). The Straub's cannula contained 2.0 ml of Ringer's solution, the composition of which was as follows: NaCl 111 m*M*; KCl 2.7 m*M*; $CaCl_2$ 1.8 m*M*; $NaHCO_3$ 1.2 m*M*; glucose 2.7 m*M*. The bathing medium was aerated via a fine polyethylene tubing inserted into the cannula. All the experiments were conducted at room temperature (20–25 °C).

3-Deoxydigitoxigenin used was synthesized and kindly supplied by Dr. M. Okada of Tokyo Biochemical Research Institute, Tokyo[4]. Digitoxigenin was obtained from the same source. Stock solutions of these two compounds were prepared, by dissolving them in 70% ethanol in a concentration of 1 mg/ml. Immediately before use, the stock solution of digitoxigenin was diluted to a desired concentration with distilled water. Because of the low water solubility of 3-deoxydigitoxigenin, the stock solution of this compound was first diluted with 70% ethanol to make a solution of 100 µg/ml, from which a solution of a desired concentration was prepared by dilution with distilled water.

Prior to the administration of the drugs, an impairment of the contractile force was induced by reducing the calcium concentration of Ringer's solution to 0.6 m*M*, $^1/_3$ of the normal. Then a small volume of the test solu-

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