and applied to 4 preparative plates for TLC. The green fluorescent band (Rf 0.47) containing purified OT_C was scraped from the developed plates and eluted with methanol. 50 mg of OT_C was recovered after the methanol was evaporated.

Ochratoxin T_A was prepared by alkaline hydrolysis of OT_C. 6/10 ml of 1 *N* NaOH was added to an OT_C solution (20 mg in 1.2 ml EtOH, the solution turned from a light pink to a faint yellow upon addition of NaOH). Upon dilution with distilled water, acidified with 1 *N* HCl, the mixture was extracted exhaustively with ethyl ether, evaporated, and then applied to four preparative TLC plates. After developing, the plates showed two fluorescent bands, one at the origin (blue) and the other with an Rf of 0.26 (greenish). The latter band was scraped from the plates, and then eluted with methanol. 14 mg of OT_A was obtained after the MeOH was evaporated.

Results and discussion. Characterization of OT_C and OT_A. Thin layer chromatography of the purified analogs at 10 µg per spot revealed only 1 round green fluorescent spot at an Rf value of 0.47 for OT_C and 1 of 0.26 for OT_A. The Rf value for OA standard was found to be 0.57 under the same conditions. Tyrosine was the only amino acid detected (Beckman Spinco Model 120B amino acid analyzer) after hydrolysis in 6 *N* HCl at 110 °C for 48 h. Ochratoxin T_A was readily converted back to OT_C upon esterification with EtOH in BF_3. Although mass spectral analysis (Associated Electrical Industries, Ltd. M-9 mass spectrometer) for OT_A was unsuccessful because of the low volatility of the product, excellent mass spectral results for OT_C were obtained (Figure). The presence of halide (chlorine) ion in the molecule was evident from the absorption intensities of a number of ion peak pairs, such as at *m/e* 447 and 449, 402 and 404, 374 and 376, 239 (base peak) and 241, having a ratio of approximately 3 to 1. The molecular weight of OT_C as determined by the mass spectrometrical method was found to be 447 (requires 447). The absorption maxima of OT_C above 300 nm resembled those of OA (λ maxima at 330 nm and 380 nm), and were further found to be dependent upon pH and environmental conditions[5]. Molar absorptivities ε (calculated on the basis of tyrosine content) for OT_C at 330 nm and 380 nm were found to be 2610 and 9660 in EtOH, and 7570 and 200 in acidic EtOH (2 ml EtOH and 1 ml 0.1 *N* HCl), while those for OT_A were found to be 9770 in EtOH (380 nm) and 7770 in acidic EtOH (330 nm). Spectrophotometrical titration[5] revealed that the dissociation constants for the phenolic hydroxyl group in the isocoumarin ring of OT_C and OT_A were 6.41 and 7.09 (compared to values of 7.14 for OC nad 7.05 for OA; ref. [5]) respectively. $[\alpha]_D^{25}$ for OT_C and OT_A in EtOH were found to be −41.2 (c. 0.28) and −141 (c. 1.3).

Chicken embryo assay. The toxicities of OT_C and OT_A were tested by chicken embryo assay as previously described, and the results are shown in the Table. Ochratoxin T_C was found to be slightly more toxic than OA, but OT_A is slightly less toxic than either of these[5]. Since OT_C has a lower pK for the phenolic hydroxyl group than does OA, whereas OT_A has a higher pK value than OA, these results support our previous postulation regarding the importance of the dissociation of the phenolic hydroxyl group in ochratoxin for intoxication. Nevertheless, studies on other animal systems will have to be carried out to pinpoint the lesions on target organs or tissues before any conclusions can be made concerning the mode of action of these new analogs[9].

Testing of culture filtrates of *Aspergillus ochraceus* and *Penicillium viridicatum*. In an attempt to find whether some ochratoxin-producing fungi might produce OT_C and OT_A, crude extracts obtained from rice in which *A. ochraceus* 3174 and *P. viridicatum* were grown for 1 week at room temperature were analyzed by TLC method. No fluorescence spots having Rf values resembling those of OT_C and OT_A were detected. It was therefore concluded that these 2 cultures, at least, do not produce these analogs in nature under the conditions described. Whether other ochratoxin producers synthesize these analogs, and whether the same organisms we tested produce these analogs when incubated under other conditions, are two questions which remain to be investigated[10].

Zusammenfassung. Zwei Ochratoxinanaloge, Ochratoxin T_A (OT_A) und T_C (OT_C) wurden aus Ochratoxin-α chemisch synthetisiert indem Phenylalanin und sein Äthylester im Molekül durch Tyrosin und Tyrosinäthylester substituiert wurden. Der Hühnerembryonentest ergab, dass OT_C etwas toxischer und OT_A etwas weniger toxisch ist als Ochratoxin A (OA).

Toxicity of OT_A and OT_C to 8-day-old chicken embryos

Analogs	Amount injected (µg/egg)	Toxicity (No. dead/No. surviving embryos, 2 weeks after injections)
OT_C	28	3/3
	14	9/10
	7	7/10
	3.5	1/10
OT_A	21	6/10
	10	1/10
	5	1/10

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Blockade of the Hydrosmotic Effect of Vasopressin by Cytochalasin B

Cytochalasin B (CB) is a macrolide antibiotic independently isolated by Turner in England[1] and by Rothweiler and Tamm[2] in Switzerland, under the name of phomin. Since the report by Carter in 1967[3], CB has been intensively investigated in many cell systems. It has been shown that this substance interacts with microfilaments, disorganizing the ectoplasmic cell web[4,5] and alters several cell functions, such as cytokinesis, motility,

endocytosis and cytoplasmic streaming[1,6]. Given the wide variety of cell phenomena affected by CB, we considered it potentially interesting to investigate the effects of this compound on sodium and water transport in amphibian epithelia, particularly after stimulation by neurohypophyseal hormones.

Quarter bladders of toads *Bufo marinus* were mounted on glass chambers designed for the study of osmotic water flow, and exposed to Ringer solutions of standard composition[7]. Measurements were performed with an automatic optical method, used previously with frog skin[8]. Taking into account the mechanical fragility of toad bladder, as compared to frog skin, and the possibility of damage of the epithelium by the supporting nylon meshes, preliminary studies were carried out to assess the suitability of the method for toad bladder. As shown in Figure 1, the bladders behaved normally under the experimental conditions, allowing for a small basal flow in the absence of neurohypophyseal hormones and reacting promptly and typically to Pitressin with a large increase in water flow.

Addition of CB to the bathing Ringer solutions resulted in a striking blockage of the hydrosmotic effect of Pitressin on toad bladder. This phenomenon is clearly illustrated in Figure 2: 2 quarter bladders exposed to CB showed no detectable increase in water flow after addition of Pitressin, whereas a 3rd quarter from the same bladder exhibited the usual response to the hormone.

Initial experiments were performed with both surfaces of the bladder exposed to 10 µg/ml of CB for 3 h. Subsequently, only the serosal surface was exposed to the drug, while the concentration and time of exposure were reduced to 1 µg/ml and 30 min respectively. In 30 experiments with CB, a total block of the water flow was observed and in 3 other experiments the inhibition ranged from 80 to 95%. As CB was dissolved in dimethylsulfoxide (DMSO), additional studies were carried out to exclude an effect of the solvent. No anomaly in the response to Pitressin was found in the presence of DMSO within the range of concentrations used in these studies, i.e., 0.01 to 1% of DMSO in Ringer solution. Furthermore, it was also observed that CB blocked transepithelial water transport when given to membranes with very high rates of water flow by previous stimulation with Pitressin.

In contrast with the blockage of the hydrosmotic effect of Pitressin, CB did not prevent the ability of the hormone to stimulate sodium transport in toad bladder. This can be seen in the lower half of Figure 3, showing the results obtained in a 4th quarter of the same bladder used in the water flow experiments of Figure 2. In a total of 10 paired experiments, the increase in sodium transport, taken as the increase in short circuit current, was 23.1 ± 5.36 µA/cm² (Mean ± S.E.) in the presence of CB and 23.0 ± 5.29 µA/cm² in the absence of the

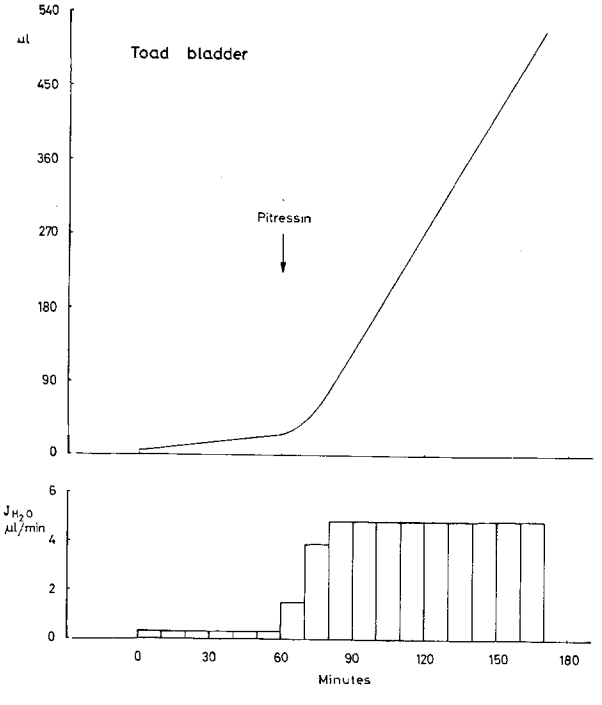

Fig. 1. Automatic recording of water flow (J_{H_2O}) with an optical method[8]. Upper half: direct recording of the displacement of the meniscus inside the pipette. Lower half: conversion to flow units. Osmotic gradient established at time 0: serosal side – normal Ringer solution; mucosal side – Ringer solution diluted 10 times.

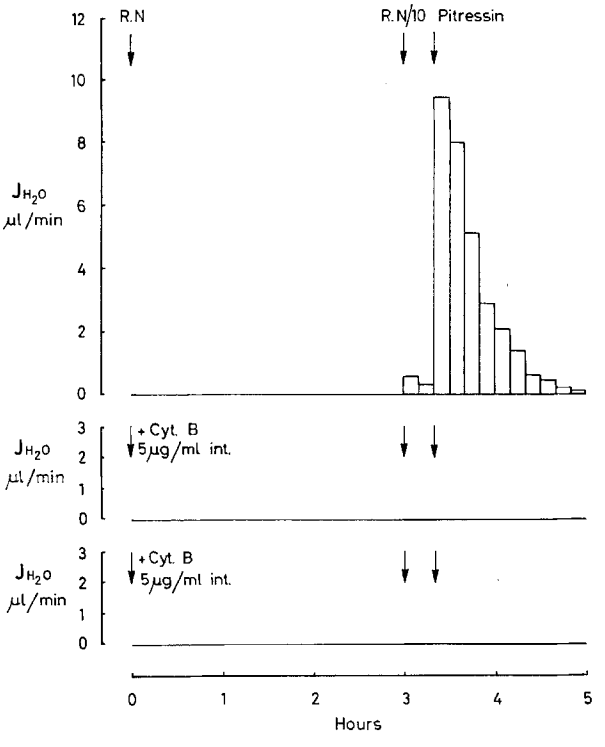

Fig. 2. Inhibition by cytochalasin B of the osmotic flow induced by Pitressin on toad bladder. The drug was added to the internal (serosal) side at time 0. RN, normal Ringer on both sides of the bladder. R.N./10, osmotic gradient as described in Figure 1. Water flows (J_{H_2O}) were determined in 3 independent quarters of the same bladder.

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macrolide. In another set of experiments, effects of CB on sodium transport were studied in a different amphibian epithelium, the ventral skin of frogs *Rana ridibunda*. As shown in the upper half of Figure 3, CB did not block the natriferic effect of oxytocin on frog skin, a result consistent with the observations made on toad bladder[9].

The studies reported here demonstrate that in the presence of CB a striking dissociation of the hydrosmotic and natriferic effects of Pitressin can be observed in toad bladder. Further experiments are needed to define the site and mode of action of the antibiotic. Two major possibilities exist: 1. CB affects primarily the permeability to water of the apical membrane of epithelial cells; and 2. CB disrupts microfilaments which play a role in the stimulus-effect coupling of neurohypophyseal hormones.

Recent reports showing that CB can directly affect membrane transport[10,11] support the first hypothesis. Concerning the second hypothesis, two aspects of toad bladder epithelial cells must be emphasized: a) a conspicuous cell web of microfilaments is present just beneath the apical membrane[12]; and b) the granular cells exhibit a great number of granules, the contents of which have recently been implicated in the hydrosmotic effect of vasopressin[13,14]. The interplay between CB, cell web and secretion of granules has been extensively studied in other systems[5], suggesting that similar phenomena might occur in toad bladder and contribute to the changes in water permeability described in this report. Given the wide variety of cell phenomena altered by CB[1,4,6], it is conceivable that more than one effect is involved in the inhibition of osmotic flow across toad bladder. In fact, in addition to alter the permeability to water of the apical membrane of the epithelial cells, CB may affect other structures, such as the submucosal smooth muscle, which is known to influence water flow across the bladder under certain experimental conditions[15].

In a report just published, TAYLOR et al.[16] described effects of CB on toad bladder very similar to those reported here, differences in the degree of inhibition being probably attributable to differences in the techniques used to measure water flow. These authors also showed that CB inhibits the hydrosmotic effect of cyclic AMP[17,18]. The concept of an interrelationship between changes in membrane permeability and secretion of granules by epithelial cells deserves further attention, since it might lead to new insight on the stimulus-effect coupling of neurohypophyseal hormones and on the role of microtubules and microfilaments on transepithelial transport processes[19,20].

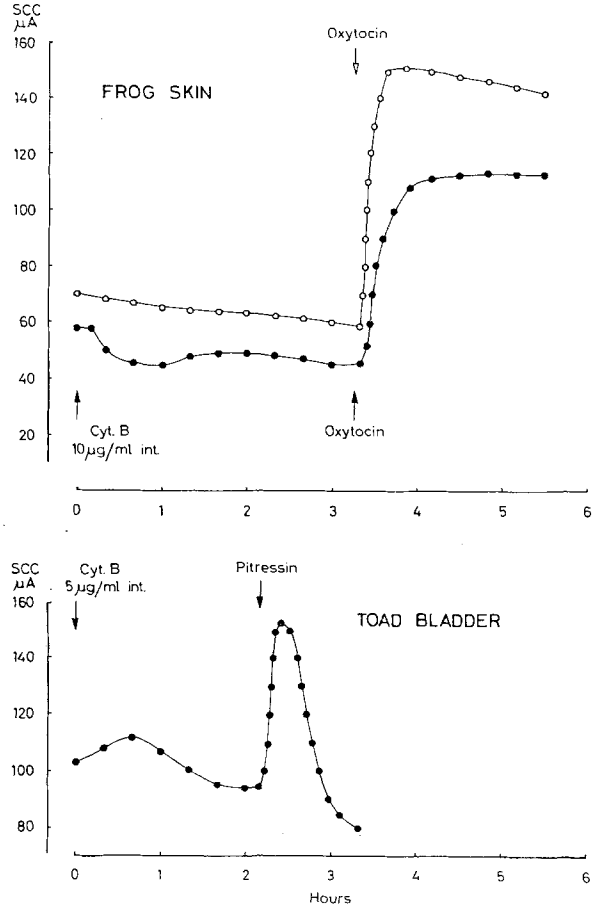

Fig. 3. Stimulation of short circuit current (SCC) by neurohypophyseal hormones in the presence of cytochalasin B added to the internal (serosal) side. Techniques for measuring SCC were described elsewhere[7]. Symbols on the upper half refer to 2 independent areas of the same skin. Lower half refers to the 4th quarter of the same bladder of Figure 2.

Résumé. La cytochalasine B dissocie les effets hydrosmotique et natriférique de la Pitressine sur la vessie de crapaud, en bloquant le transport d'eau sans pour autant modifier la stimulation du transport de sodium. Les interactions possibles entre le macrolide, les microfilaments et les altérations de perméabilité sont discutées.

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