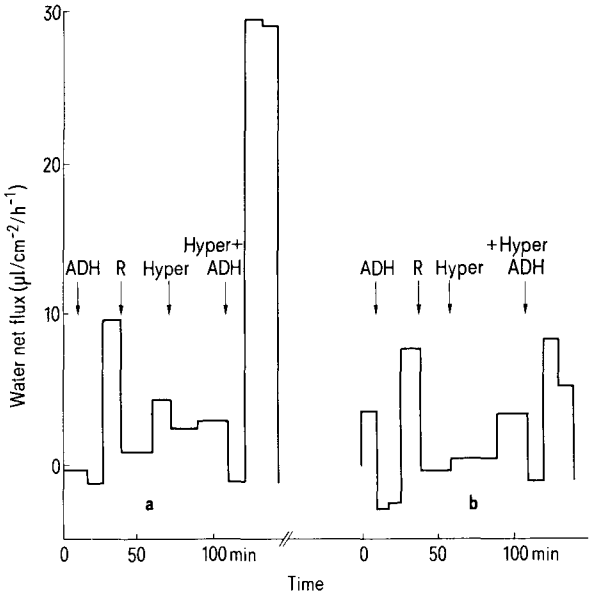
Colchicine Dissociates the Toad (*Bufo arenarum*) Urinary Bladder Responses to Antidiuretic Hormone and to Serosal Hypertonicity

The distal collecting tubule of the mammalian kidney is surrounded by an interstitium which has an osmolarity 5 to 8 times greater than that of plasma. The urinary bladder of amphibia can be regarded as a model of this structure in which the action of antidiuretic hormone (ADH) and the role of osmolarity on water permeability can be studied. Oxytocin and an increase in serosal osmolarity[1] induce an osmotic water flux in toad urinary bladder. It has been suggested that the action of hypertonicity is elicited via biochemical steps also involved in the ADH effect[2,3] and that the mucosal border of the epithelial cells would be the final site of action of both stimuli[2,4]. The existence of a common final mechanism is also supported by the inhibition of ADH and hypertonicity responses following addition of lanthanum to the mucosal bath[5]. In view of the type of interaction with noradrenaline[2], prostaglandins[2] and zinc[3] hypertonicity would enter the chain of events triggered by ADH in a post-cyclic AMP step.



Net water flux across toad urinary bladder: The effect of colchicine on the potentiation by ADH (10^{-10} *M*) of the response to a mild hypertonic serosal medium (hyper 50 m*M* sucrose added to the saline). a) control; b) experimental hemibladders (10^{-4} colchicine).

Colchicine reduces the hydrosmotic response to ADH[6,7] and it is also known that copper inhibits the response to ADH at concentrations that do not suppress the effect of hypertonicity[8]. We report here the action of copper and colchicine on the responses elicited by ADH and medium hypertonicity in toad urinary bladder.

Material and methods. The unidirectional mucosal-to-serosal flux was measured by exposing everted bladder sacs of South American toads (*Bufo arenarum*) to saline-containing tritiated water for 40 sec[9]. Net water flux was determined gravimetrically, using paired hemibladders mounted as non-everted sacs[10]. The saline solution contained (m*M*): NaCl 111; KCl 3.35; $CaCl_2$ 2.70; $NaHCO_3$ 4.0; (pH: 7.6). When the net flux was measured, the sacs were filled with a diluted (1:10) Ringer. The serosal bath was stirred by air bubbles. Oxytocin (Pitocin, Parke Davies), colchicine (Sigma), $CuSO_4$ (Merck) and sucrose were added only to the serosal bath, to final concentrations of 10^{-8}, 10^{-4}, 10^{-4} and 0.22 *M* respectively. When colchicine was tested, the bladders were incubated 4 h before being treated with ADH or hypertonicity. Results are expressed as the mean difference and standard error for paired hemibladders and analyzed by the Student *t*-test.

Results and discussion. Colchicine did not modify the unidirectional water movement at rest: in 14 experiments this drug had no significant effect on the mucosal to serosal flux (control- 443 µl.cm^{-2}.h^{-1}; experimental: 415 µl.cm^{-2}.h^{-1}; mean difference: -30 ± 34 µl.cm^{-2}.h^{-1}; p 0.1).

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Hydrosmotic response of toad urinary bladder to oxytocin and hypertonicity. Effect of colchicine and copper

Drugs tested	*n*	Water net flux (µl/cm^{-2}/h^{-1})			
		Basal	Stimulated	Inhibition (%)	*P*
ADH	11	2.07 ± 0.33	55.78 ± 8.09	57.86 ± 8.23	0.01
ADH + colchicine		1.52 ± 0.35	24.47 ± 4.90		
Hypertonicity	11	1.36 ± 0.08	37.88 ± 4.29	15.17 ± 8.60	n.s.
Hypertonicity + colchicine		1.27 ± 0.10	30.01 ± 2.46		
ADH	5	0.96 ± 0.40	28.78 ± 5.01	95.08 ± 3.85	0.001
ADH + colchicine + Cu^{2+}		1.26 ± 0.78	1.37 ± 1.86		
Hypertonicity	6	1.66 ± 0.20	36.78 ± 5.05	0.47 ± 13.90	n.s.
Hypertonicity + colchicine + Cu^{2+}		1.14 ± 0.14	34.12 ± 4.02		

Values represent the mean ± standard error. The significance was obtained from Student's *t*-test for paired data.

The hydrosmotic response to medium hypertonicity was not decreased significantly by colchicine but the effect of oxytocin, in agreement with previous results[6,7], was strongly reduced (Table). When copper was added to colchicine-treated bladders, a complete disociation between the response to ADH and hyperosmolarity was observed: while copper inhibits completely the response to ADH, it does not affect significantly the response to hypertonicity (Table).

Hypertonicity potentiates the effect of ADH[2]. To see the action of colchicine on this potentiation, a first stimulation with 10^{-10} *M* oxytocin was followed, after washing, by addition of 50 m*M* sucrose to the serosal bath. 30 min after the increase of serosal osmolarity, a second stimulation with 10^{-10} *M* oxytocin was superimposed to hypertonicity. It can be seen in the Figure that the clear potentiation observed in the control hemibladders is completely prevented in the colchicine-treated ones.

Two hypotheses have been postulated to explain the effects of colchicine[6] and cytochalasine B[11] in toad urinary bladder: 1. These alkaloids affect primarily the permeability to water of the apical membrane of epithelial cells; 2. They disrupt microtubules and/or microfilaments which would play a role in the coupling between the cyclase system and the change in membrane permeability.

Our results indicate that, in contrast to what happens with ADH, the mechanisms involved in the hydrosmotic response to hypertonicity are not altered by colchicine. If, as supported by strong experimental evidence[2], ADH and the elevation of medium tonicity induce similar changes in the mucosal border of epithelial cells, (perhaps envolving an endocytotic process[12], it seems logical to conclude that colchicine is acting at one of the steps previous to the change in membrane permeability. Since colchicine inhibits the action of exogenous cyclic-AMP[6], the effect should be located in the system which couples the nucleotide concentration with the hypothetical change in membrane structure, i.e. a post cyclic-AMP step.

It has recently been reported that in renal epithelial cells colchicine binds mainly to the cytosol fraction[13]. This reinforces the view given here of a non-membrane action of this alkaloid. A direct effect of colchicine on the apical membrane would be conceivable only if there were two permeability barriers, one triggered by ADH and the other by hypertonicity.

Resumen. Los efectos de la ocitocina y la hipertonía serosa sobre la respuesta hidrosmótica de la vejiga urinaria del sapo pueden ser disociados empleando colchicina, y mas evidentemente cuando el alcaloide es colocado junto con Cu^{++}.

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Evolution of the Genome and Cell Sizes in Salamanders

The question of the meaning of the increases in the genome size, which seem to have characterized the phylogeny of Eucaryotes, has given rise to various hypotheses which alternatively place greater weight on the evolutionary[1] or functional aspects of this problem[2]. In some groups of organisms displaying broad variations at the interspecific level, there is some correlation between the genome size and several cytological, physiological or ecological factors subjected to natural selection; hence in various cases the genome size is likely to take on an adaptive meaning[3].

Among vertebrates, the highest interspecific DNA differences are found in the Caudates (Amphibia) in which they range from 30 to over 160 (or 200, according to some authors) picograms per nucleus (pg/N). As in the lungfish, some species in this order possess the highest DNA amounts in the subphylum[4-6]. Hence, these Amphibians are suitable for the study of the pattern of correlation between the DNA amount and cell size, the latter being more manifestly variable according to its adaptive function (some workers maintain that the genome size may depend upon the cell size[7], but there is evidence that the reverse is probably true[8]).

In the present work we have compared the nuclear DNA content with the main morphometric parameters of the cell in 39 species, belonging to all the 8 families of Caudates and possessing DNA amounts which cover practically the whole range of genome variations in this order.

One of us[6] had already measured histophotometrically the erythrocyte nuclear DNA (blocked in G_1[9]). In smears, erythrocytes take on the shape of a cylinder with an ellyptical base[10]. We have measured the diameters of the ellypse by means of a Leitz screw micrometer eyepiece (15 cells for each species) and the cylinder thickness (which was seen to be constant in each smear) by means of a Horn-Gren (Leitz) microinterpherometer in monochromatic light at 546 nm, according to the 'two embedding media' method[11] (10 measurements per cell, 6 cells per species).

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