occurring at the highest phosphate concentration tested (0.3 M).

In agreement with Tapley[4], we found that there was difficulty in reproducing mitochondrial swelling results found by others, Our data on mitochondrial swelling, obtained using the same methods described by NiFhaolain and O'Donovan were not in concert with theirs[2] – agreeing more closely with Tapley's results[4]. While NiFhaolain and O'Donovan report very little swelling with 0.3 M sucrose, we found, that at this sucrose concentration, extensive swelling of rat kidney mitochondria occurred even to the point that swelling in 0.3 M sucrose was not significantly different from the swelling obtained at 0.3 M phosphate ($p > 0.05$). Why there was a difference in swelling between experiments is less important than finding no correlation between mitochondrial swelling and ammonia production. Our data do not negate the hypothesis of Guha and Chakravarti[3] that some swelling of mitochondria is needed for phosphate activation of PDG, for under every condition that we studied PDG, there was some mitochondrial swelling.

To further disassociate mitochondrial swelling from glutamine ammoniagenesis, the ΔOD's for control and acidotic rat kidney mitochondrial suspensions at 0.3 M phosphate concentrations from all experiments were plotted against ammonia production (figure). No correlation could be found between ΔOD and ammonia production when the concentration of phosphate was unchanged. In the presence of 0.3 M phosphate and 10 mM EDTA (6 flasks) mitochondrial swelling was inhibited compared to mitochondria incubated in the phosphate alone (table 2) ($p < 0.025$). Despite decreased mitochondrial swelling, no inhibition of ammoniagenesis was demonstrated.

These data do support the suggestion of NiFhaolain and O'Donovan[2] that increased permeability of mitochondrial membranes to glutamine is not the rate limiting factor for PDG activity, again because at any given phosphate concentration ammonia production from glutamine is not related to swelling (figure). We conclude that the major increase in PDG activity in acidosis is not related to increased anion permeability produced by mitochondrial swelling.

Colchicine inhibition of ADH effect on frog skin permeability*

M. Svelto and C. Lippe

Institute of General Physiology, University of Bari, via Amendola 165A, I-70126 Bari (Italy), 8 August 1977

Summary. ADH and AMPc enhance both thiourea unidirectional fluxes in frog skin. This effect is completely abolished by colchicine pretreatment. The ADH increase of thiourea discharge with or without colchicine led us to suppose that colchicine does not directly affect ADH action on outer membrane permeability, but exerts its effects on a site which is limiting for the ADH action on transepithelial permeability.

It is well known that some antimitotic agents such as colchicine inhibits certain cytoplasmic phenomena such as intracellular movement[1] and exocytotic transport[2]. These effects are due to a microtubule disruption wich occurs after a long time-lag.

Recently Taylor et al.[3] have reported that colchicine treatment strongly inhibits the action of vasopressin on osmotic water movement across the toad bladder, without affecting Na active transport. In a previous paper[4], we have demonstrated that noradrenaline-induced secretion of nonelectrolytes through the frog skin is suppressed by colchicine treatment.

This work deals with the effects of colchicine on ADH-activated thiourea permeability across the frog skin (*Rana esculenta*). Table 1 reports the effects of ADH on thiourea transepithelial fluxes. It can be seen that ADH treatment results in a symmetrical increase of the thiourea fluxes. 4 h preincubation in the presence of colchicine completely abolishes the ADH effect on both fluxes. Colchicine added immediately after the equilibration period (i.e. without preincubation) fails to influence the ADH effect on thiourea permeability.

Thus we can exclude the possibility of the inhibitory effect of colchicine being due to its interaction with the ADH receptor. The presence of a wide time-span seems to confirm that the colchicine effect is due to a true interaction with microtubule protein[5]. Lumicolchicine, a colchicine derivative which does not bind microtubule protein, is generally used as a control of the specificity of colchicine effect[5].

In frog skin, 4 h exposure to lumicolchicine does not affect the ADH action on thiourea permeability. Thus the presence of a wide time-span for the colchicine effect, and the failure of lumicolchicine to affect ADH action, strongly suggest that colchicine effect is quite specific, i.e. it is due to interaction with microtubule protein. Dibutyryl-AMPc 10^{-3} M mimics the ADH effect on thiourea permeability (table 2), this effect being completely abolished by colchicine pretreatment. Cyclic AMP is generally considered the cellular mediator of ADH actions. Thus, the inhibition induced by colchicine appears to be subsequent to cAMP production. Finally the effect of colchicine on ADH action cannot be due to tissue damage[4]. In fact thiourea control values (table 1 and 2) are virtually the same with and without colchicine.

All these considerations strongly support the idea that colchicine inhibition of ADH action on thiourea permeability is related to the disruptive effect of the antimitotic on microtubules. It is very difficult to propose a model for this ADH action, as the existence of a symmetric ADH effect, colchicine-sensitive, seems to exclude a secretory process mediated by exocytotic vesicles. However, it is improbable that ADH effect is mediated by a microtubule assembly only, because the hormone effect is very selective.

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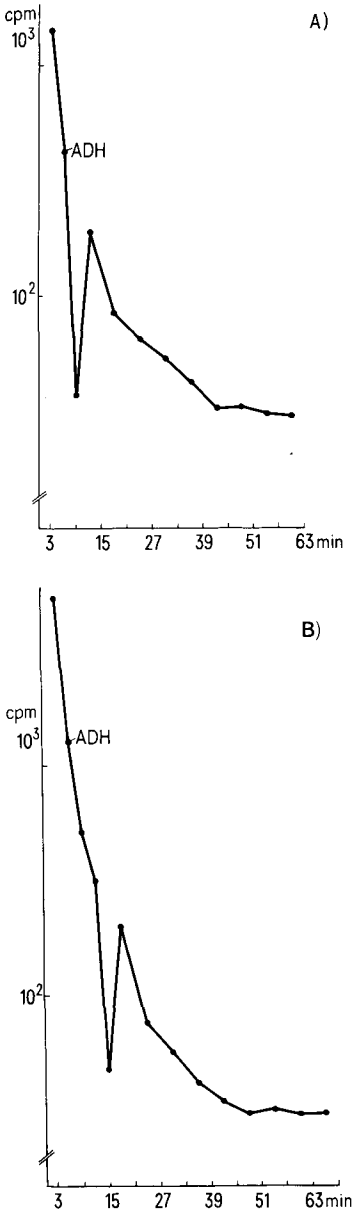
In fact, in frog skin ADH increases the permeability of thiourea, but not that of glycerol, a molecule with a similar radius and lipid solubility (unpublished observations). ADH effect on nonelectrolyte permeability is present in toad bladder as well and is localized at the outer plasma membrane of epithelial cells, thus allowing extension of localization to include the frog skin[6]. It is possible that colchicine does not directly affect ADH action but rather impairs some mechanism which conditions the hormonal effect. Such a mechanism should be based on intercellular communication.

Recently Mills et al.[8] have proposed a model for Na transport for the frog skin, involving intercellular communication between different cell layers. Such a communication should be a limiting step for ADH action on thiourea transepithelial permeability. In order to test this hypothesis, we have performed experiments with the washing out technique. Results are reported in the figure. ADH addition is seen to result in a abrupt increase of thiourea discharge. This effect is also present in the skin colchicine treated. These experiments seem to support our hypothesis that colchicine does not directly affect ADH action on outer membrane permeability but influences some mechanism which exerts a permissive role in ADH action on transepithelial permeability. Most probably this effect may be located at the intercellular junctions.



Kinetic of thiourea washout from preloaded frog skin. Symmetrical halves of frog skin were loaded with 5 μCi/ml ^{14}C-thiourea: *A* served as control, *B* was preincubated for 4 h in the presence of $2 \cdot 10^{-5}$ M colchicine. Washout towards the external medium was conducted for 2 3-min periods; afterwards ADH (50 mU/ml) was added to the inner bathing fluid and the experiment was carried out for several periods.

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Table 1. Transepithelial fluxes of thiourea 1 mM across frog skin under ADH treatment

Experimental conditions	n		A	B	Δ
Control	6	ϕ_o	20.2 ± 1.5	37.7 ± 2.6	15.4 ± 1.7
		ϕ_i	19.2 ± 2.7	32.0 ± 3.5	12.7 ± 1.6
$2 \cdot 10^{-5}$ M colchicine preincubation for 4 h	6	ϕ_o	19.2 ± 1.8	20.9 ± 1.8	1.8 ± 1.4
		ϕ_i	16.8 ± 1.7	19.8 ± 1.8	2.9 ± 2.4
$2 \cdot 10^{-5}$ M colchicine added on B	6	ϕ_o	23.9 ± 2.9	42.1 ± 5.9	18.1 ± 3.0
		ϕ_i	24.8 ± 2.6	41.4 ± 2.1	16.3 ± 2.2
$2 \cdot 10^{-5}$ M lumicolchicine preincubation for 4 h	5	ϕ_o	30.5 ± 1.6	45.9 ± 3.2	15.4 ± 2.0

Thiourea transepithelial fluxes measurements were carried out according to the method previously reported[7].
A = fluxes before ADH treatment; B = fluxes after 2 h of ADH (50 mU/ml in the inner fluid) treatment. Δ = B–A. ϕ_o = flux towards the external medium and ϕ_i = flux towards the internal medium expressed in nmoles $\cdot 10^{-1}$ cm^{-2}h^{-1}. Values are mean ± SE. n = number of experiments.

Table 2. Transepithelial fluxes of thiourea across frog skin under dibutyryl cyclic AMP treatment

Experimental conditions	n		A	B	Δ
Control	6	ϕ_o	24.5 ± 2.5	38.2 ± 2.5	13.7 ± 2.9
		ϕ_i	20.2 ± 1.2	39.9 ± 3.0	19.8 ± 3.2
$2 \cdot 10^{-5}$ M cholchicine preincubation for 4 h	9	ϕ_o	22.3 ± 2.2	20.9 ± 4.2	0.9 ± 2.1
		ϕ_i	23.9 ± 2.6	28.2 ± 3.2	3.9 ± 1.6

A = fluxes before cyclic AMP treatment; B = fluxes in the presence of dibutyryl cyclic AMP 10^{-3} M. Δ = B–A.
ϕ_o = flux towards the external medium and ϕ_i = flux towards the internal medium expressed in nmoles $\cdot 10^{-1}$ cm^{-2} h^{-1}. Values are mean ± SE. n = number of experiments.