obtained 6 cell generations in 18 h (1). Phenylalanine is an essential amino acid for *Tetrahymena*[7,8] and if it is omitted cell multiplication ceases (2). The cells go through less than 1 doubling in 18 h if the phenylalanine concentration is redued to 0.1 mM (3). If all the other 16 amino acids are reduced to a similar level the cells produce 4 doublings (4). We interpret the results shown in (3) and (4) as follows: amino acids present in the standard synthetic medium block (competitively and otherwise[9]) uptake of phenylalanine in low concentrations (3); this idea is supported by the finding that reductions in the concentrations of all of the amino acids to levels similar to that of phenylalanine result in good growth (4). If phenylalanine is offered solely as 0.05 mM phenylalanyl-phenylalanine (5) or solely as 0.1 mM phenylalanine-leucine-containing dipeptides in nutrient media having high amino acid concentrations (6) and (7) the cells grow well. (8) shows that 0.1 mM leucine – which would be formed if the peptides in (6) and (7) were fully hydrolyzed before uptake – does not in itself stimulate cell multiplication.

These results show that high external amino acid concentrations affect the utilization of free phenylalanine (3) and phenylalanine-containing di-peptides (5)-(7) in different ways. This suggests that the cells take up free phenylalanine and di-peptides by different mechanisms. This suggests that the di-peptides are not hydrolysed before uptake, or, in other words, that the di-peptides are taken up intact. We want to point out that evidence from bacteria and mucosa cells indicate that these cells have only one site responsible for the uptake of all di-peptides[2,5].

In these experiments we used the inbred strain DIII of *T. thermophila*[10]. The experiments were repeated with inbred strain BV and with *T. pyriformis* with essentially the same results (not shown). We also obtained similar results with the temperature-sensitive, food-vacuoleless mutant, NP1[11]. We therefore believe that peptide uptake is independent of the food vacuole membrane. Unfortunately, no mucocyst-less mutant cell line of *Tetrahymena* has been isolated yet. Therefore we cannot at the present time answer the question whether or not these organelles play any role in the uptake of peptides.

We want to point out that the occurrence of peptidases in the cytoplasm and on the plasma membrane of *Tetrahymena*[12], makes it difficult to establish in a more direct way whether these cells take up di-peptides.

Cell multiplication in cultures of *Tetrahymena thermophila*

| Nutrient medium | No. of cell generations obtained after 18 h |
|---|---|
| (1) SNM complete | 6 |
| (2) SNM minus Phe | 0 |
| (3) SNM minus Phe plus 0.1 mM Phe | < 1 |
| (4) SNM all amino acids at 0.1 mM | 4 |
| (5) SNM minus Phe plus 0.05 mM Phe · Phe | 4 |
| (6) SNM minus Phe plus 0.1 mM Leu · Phe | 4 |
| (7) SNM minus Phe plus 0.1 mM Phe · Leu | 4 |
| (8) SNM minus Phe plus 0.1 mM Phe plus 0.1 mM Leu | < 1 |

SNM: synthetic nutrient medium[13]; Phe: L-phenylalanine; Leu: L-leucine; Phe · Phe: phenylalanyl-phenylalanine; Leu · Phe: leucyl-phenylalanine; Phe · Leu: phenylalanyl-leucine. Incubation temperature: 37 °C; initial population density: 5000 cells/ml; the cell: *Tetrahymena thermophila*, inbred strain DIII. Source of inoculum: cells transferred to phenylalanine-free SNM for 24 h.


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## Vanadate: non-selective inhibition of transepithelial transport of $Na^+$, $H^+$ and water[1]


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*Summary.* In the isolated urinary bladder of the toad, $10^{-5}$–$10^{-4}$M orthovanadate produces inhibition of the active transport of $Na^+$ and $H^+$ ions as well as of antidiuretic hormone-mediated osmotic flow of water. Since transport of $H^+$ ions and osmotic water flow are not inhibited when ($Na^+ + K^+$)-ATPase is inhibited by ouabain, biological actions of vanadate are not necessarily related to inhibition of ($Na^+ + K^+$)-ATPase.


Observations that nano- to micro-molar concentrations of orthovanadate ($VO_4\equiv$) inhibit ($Na^+ + K^+$)-ATPase activity[2–4] resulted in numerous publications confirming inhibition of sodium and/or potassium transport in a variety of tissues or organs, including red blood cells[4] squid axon[5] and kidney[6–8]. In the latter organ, in vitro vanadate increased urine flow more than 10-fold and the rate of urinary excretion of $Na^+$ several 100-fold with only a minor increase in $K^+$ excretion[6,7] and a decrease in urinary concentration of solutes[8]. Recently, De Sousa and Grosso[9] reported that $10^{-4}$–$10^{-3}$M metavanadate ($VO_3^-$) produced inhibition of sodium transport and cyclic-AMP-induced osmotic water flow in amphibian epithelia. The present report confirms the finding that vanadate inhibits $Na^+$ transport and water flow but also demonstrates that $10^{-5}$–$10^{-4}$M orthovanadate inhibits urinary acidification (putatively mediated by $H^+$ transport).

*Methods.* Urinary bladders were removed from doubly pithed toads, *Bufo marinus*, and incubated in an amphibian Ringers solution[11] containing 2.4 mM bicarbonate. Active

transport of sodium was quantitated by the short-circuit current (SCC) technique[10] in media equilibrated with air, pH 8.4. When the $K^+$ concentration of the solution was varied, reciprocal changes in sodium were made so that total monovalent cation concentration and osmolality were unchanged. The osmotic flow of water across hemibladders, incubated as bags, was determined gravimetrically[16]. Urinary acidification was quantitated by the reverse short-circuit current (RSCC) technique[11] with the media equilibrated with 99% $O_2$–1% $CO_2$, pH 7.2.

*Results.* SCC was inhibited by orthovanadate. As shown in table 1, experiment A, 50% inhibition of SCC was produced by $10^{-5}$M vanadate whether the concentration of $K^+$ in the serosal solution was 3.5 mM or 35 mM. As shown in table 1, experiment B, when SCC was pre-inhibited 56±4% by the addition of $5\times10^{-7}$M amiloride to the mucosal solution, vanadate inhibited SCC to the same extent as in tissues not exposed to amiloride.

The osmotic flow of water produced by 100 mU/ml antidiuretic hormone was measured when $(Na^+ + K^+)$-ATPase was inhibited by either $10^{-3}$M ouabain (serosally) or $5\times10^{-5}$M vanadate (serosally). Flow of water along a gradient of 55 mOsm/kg $H_2O$–220 mOsm/kg $H_2O$ was 1.01±0.13 g·(30 min)$^{-1}$ in ouabain-treated tissues but was only 0.32±0.04 g·(30 min)$^{-1}$ in the presence of vanadate ($n=4$, $p<0.025$). Similar degrees of inhibition of water flow were produced when vanadate treated tissues were compared with untreated controls.

Urinary acidification, presumed to occur secondary to extrusion of $H^+$ across the apical plasma membrane, was also inhibited by orthovanadate. As shown in table 2, $10^{-4}$M vanadate in the serosal solution inhibited RSCC by 43±12% ($p=.005$).

*Discussion.* The inhibition of sodium transport by vanadate (table 1) was predicted from prior work on $(Na^+ + K^+)$-ATPase. However, the lack of effect of external $K^+$ concentration (table 1, experiment A) was not expected. Since inhibition of $(Na^+ + K^+)$-ATPase by vanadate may be antagonized by $Na^+$[11], amiloride was used to decrease the entry of $Na^+$ into the cell[13-15] and, therefore, presumably decrease the intracellular concentration of $Na^+$. However, the inhibition of sodium transport produced by vanadate was not altered (table 1, experiment B). Although changing extracellular $K^+$ and intracellular $Na^+$ did not produce the anticipated alterations in orthovanadate-mediated inhibition of sodium transport, it is still possible that SCC was inhibited secondarily to inhibition of $(Na^+ + K^+)$-ATPase by orthovanadate, because the interaction of $Na^+$, $K^+$ and $VO_4\equiv$ on the ATPase of toad urinary bladder has not been examined.

However, it is clear that orthovanadate produces alterations in epithelial function other than by inhibition of $(Na^+ + K^+)$-ATPase. The inhibition of osmotic flow of water produced by vanadate was not mimicked by inhibition of $(Na^+ + K^+)$-ATPase with $10^{-3}$M ouabain. Thus, we confirm that vanadtae has an action on antidiuretic-hormone-stimulated water permeability[9].

Finally, urinary acidification is conducted by the urinary bladder of the toad even when $Na^+$ transport is blocked by either mucosal amiloride or serosal ouabain[11]. Thus, urinary acidification does not require a functional $(Na^+ + K^+)$-ATPase. However, as shown in table 2, orthovanadate produced inhibition of RSCC, a quantitative measure of urinary acidification[11].

These studies demonstrate that $10^{-5}$–$10^{-4}$M orthovanadate in the serosal solutions inhibited 3 transepithelial transport functions in the isolated urinary bladder of the toad - $Na^+$ transport, antidiuretic-hormone-induced water flow and $H^+$ transport. Only the 1 of these is inhibited when $(Na^+ + K^+)$-ATPase is inhibited by ouabain[11,17]. Since vanadate is an inhibitor of a variety of transport functions in epithelia, it would be hazardous to assume that biological actions of vanadate on transport functions are necessarily mediated via inhibition of $(Na^+ + K^+)$-ATPase.

Table 1. Vanadate inhibition of short-circuit-current

| Experiment A $[VO_4\equiv]$ | SCC (percent of control) $[K^+]=3.5$ mM | $[K^+]=35$ mM |
|---|---|---|
| $10^{-6}$ M | 122± 7 | 105±6 |
| $5\times10^{-6}$ M | 100±12 | 83±8 |
| $10^{-5}$ M | 50± 8 | 54±7 |
| $10^{-4}$ M | 24± 4 | 23±5 |
| **Experiment B** $[VO_4\equiv]$ | **SCC (percent of control)** Control | Amiloride |
| $5\times10^{-6}$ M | 93±6 | 105±12 |
| $10^{-5}$ M | 74±6 | 84± 5 |
| $10^{-4}$ M | 30±5 | 36± 3 |

SCC was measured in matched quarterbladders from 8 animals (A) or 4 animals (B). All values are means±SEM. In A, orthovanadate was added to the serosal solutions at the indicated concentrations at 60-min-intervals. The resulting SCC is expressed as a percent of the SCC present in control tissues exposed to the same concentration of $K^+$ but no vanadate. In B, $5\times10^{-7}$ M amiloride was added to the mucosal solution of half of the quarterbladders. Vanadate was added serosally at 60-min-intervals.

Table 2. Vanadate inhibition of reversed short-circuit-current

| $[VO_4\equiv]$ | Control RSCC (percent of control) | Vanadate | Vanadate control |
|---|---|---|---|
| $5\times10^{-6}$ M | 88± 5 | 86±8 | 0.98±0.075 |
| $10^{-5}$ | 76± 9 | 65±9 | 0.89±0.12 |
| $10^{-4}$ | 62±14 | 31±5 | 0.57±0.12* |

RSCC was measured in paired hemibladders from 8 animals. The RSCC at the start of the experiment was 11.0±1.0 μA. Sodium orthovanadate was added to the serosal solutions to the indicated final concentrations at 60-min-intervals. The ratio of the percent of RSCC remaining in the vanadate-treated tissues, divided by the percent of RSCC remaining in the control tissues is shown in the right hand column. * $p=0.005$.

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