

(see Chiarelli<sup>5</sup> for the references to the papers where the original data have been published).

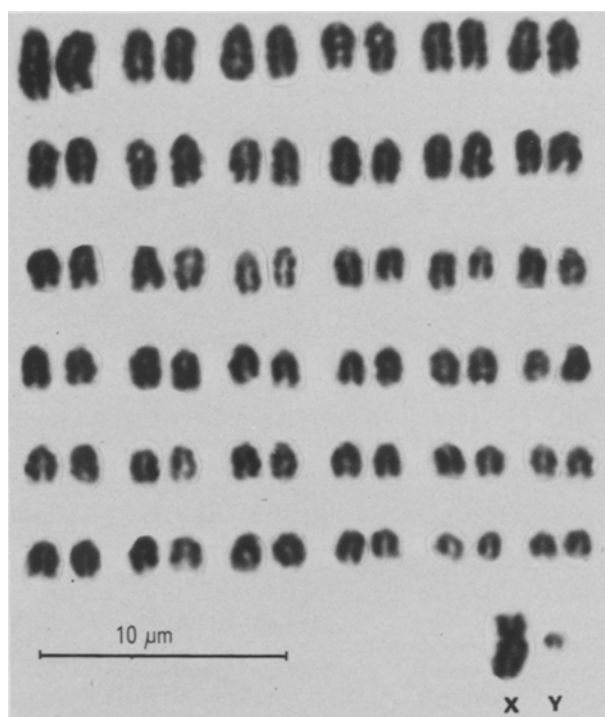
In this table we can see that 6 species have in common  $2n=74$ . Only 2 species, *C. brachyurus* and *A. microtis*, present differences in diploid number. In the first one, an extra pair is present and in the latter 2 extra very small chromosomes (microchromosomes) occur in 1 of the 2 specimens investigated by Wurster and Benirschke<sup>9</sup>. Apart from this small variation the diploid number is the same in all other species, in spite of the great morphological differences existing among them. These are very clear when *Pseudalopex gymnocercus* is compared with *Speothos venaticus*, 2 species that also occupy very different ecological niches.

The karyotype of *Cerdocyon thous*, although keeping  $2n=74$ , is rather aberrant, showing 34 metacentric autosomes and the highest NF (110) among the carnivora. How this high NF has been reached is an interesting point to be investigated. Perhaps it originated by pericentric inversions

such as have been clearly established in the genus *Peromyscus* (Hsu and Arrighi<sup>10</sup>), or by acquisition of heterochromatic 'second arms' as is also found in the same genus (Duffey<sup>11</sup>, Pathak et al.<sup>12</sup>) and in *Uromys* (Baverstock et al.<sup>13</sup>). It would be necessary to perform C-banding techniques on the chromosomes of *Cerdocyon thous* to confirm this assumption.

The uniformity of the karyotype of the South American canids, however, is not so striking as in the group of *Canis s.s.* and the allied genus *Lycaon*, where the  $2n$  is 78 and the NF is always 80, even in the high polymorphic domestic dog. On the other hand, the South American group does not show the high karyotypical diversity observed in the genus *Vulpes* and related genera (*Otocyon*, *Urocyon* and *Fennecus*). So the  $2n$  and the NF of the South American canids are higher than in *Vulpes* and lower than in *Canis* having as a group their own karyotypical characteristic. The South American group including *P. gymnocercus* also lack the 'marker chromosomes', a generalized characteristic in the carnivora and an ancestral character in the Canidae, which is present in the genus *Vulpes* (Wurster and Benirschke<sup>9</sup>).

A question that remains to be worked out is the degree of intraspecific variability of the karyotype in this group of canids since very few individuals of each species have been studied. It would be also interesting to know what degree of homology exists between the particular chromosomes of *Chrysocyon* and *Cerdocyon* and the karyotypes of the species with a common NF=76; this could be investigated with banding techniques.



Karyotype of male *Pseudalopex gymnocercus*.

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## Evidence for di-peptide uptake in *Tetrahymena*

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**Summary.** We have established growth conditions in a synthetic nutrient medium in such a way that utilization of free phenylalanine, but not of phenylalanine-containing di-peptides, limits cell multiplication in species of the genus *Tetrahymena* (Ciliata). These results suggest that these cells take up intact di-peptides.

How widespread is the occurrence of peptide uptake in pro- and eukaryote cells? So far, it has been established only in certain bacteria<sup>1,2</sup>, yeast<sup>3</sup>, barley seeds<sup>4,5</sup> and mucosa cells<sup>5,6</sup>. The results to be presented here suggest that it also occurs in species of the ciliate protozoon *Tetrahymena*.

Using growth conditions and cell counting procedures described elsewhere we obtained the results for *Tetrahymena* multiplication shown in the table. In a synthetic nutrient medium in which 17 amino acids (including phenylalanine) were present in concentrations around 2 mM each, we

obtained 6 cell generations in 18 h (1). Phenylalanine is an essential amino acid for *Tetrahymena*<sup>7,8</sup> 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 reduced 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<sup>9</sup>) 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<sup>2,5</sup>.

In these experiments we used the inbred strain DIII of *T. thermophila*<sup>10</sup>. 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<sup>11</sup>. 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*<sup>12</sup>, 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<sup>13</sup>; 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<sup>+</sup>, H<sup>+</sup> and water<sup>1</sup>

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

Observations that nano- to micro-molar concentrations of orthovanadate (VO<sub>4</sub><sup>3-</sup>) inhibit (Na<sup>+</sup> + K<sup>+</sup>)-ATPase activity<sup>2-4</sup> resulted in numerous publications confirming inhibition of sodium and/or potassium transport in a variety of tissues or organs, including red blood cells<sup>4</sup> squid axon<sup>5</sup> and kidney<sup>6-8</sup>. In the latter organ, in vitro vanadate increased urine flow more than 10-fold and the rate of urinary excretion of Na<sup>+</sup> several 100-fold with only a minor increase in K<sup>+</sup> excretion<sup>6,7</sup> and a decrease in urinary concentration of solutes<sup>8</sup>. Recently, De Sousa and Grosso<sup>9</sup>

reported that 10<sup>-4</sup>–10<sup>-3</sup>M metavanadate (VO<sub>3</sub><sup>3-</sup>) 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<sup>+</sup> transport and water flow but also demonstrates that 10<sup>-5</sup>–10<sup>-4</sup>M orthovanadate inhibits urinary acidification (putatively mediated by H<sup>+</sup> transport).

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