dark stained vesicles, which also appear after inhibition, might be due to the presence, in some cytoplasmic inclusions, of phosphates[24] which, as observed by Lonnerholm[18], precipitate independently of CA activity, as well as carbonates[24] which might have analogous behaviour. Alternatively, they might also be due to an extraordinarily high enzyme concentration. The results obtained show that CA is present in Malpighian tubules of *Culex pipiens*. In particular, our observations show that CA activity is present on the membranes of the cytoplasmic inclusions (figure 2). The localization of this enzyme on cellular surfaces, on the other hand, has been observed already, at ultrastructural level, on the microvilli of the parietal cells in the gastric mucosa of rat and mouse[12,19,25]. No evidence of CA presence has been found at the level of the cellular membranes.

These results agree with what is known both about the enzyme function and about the characteristics of cytoplasmic inclusions, described by Berkaloff[26] and Smith[27]. In fact CA has been localized in the shell glands of birds' eggs and in the glands which produce the shell in molluscs[2], which means that this enzyme is involved in the mechanism of calcium deposition. Calcium is present in many granular formations of the cytoplasmic inclusions in Malpighian tubules[26].

Moreover the vesicles of the Malpighian tubule cells are responsible for the transfer, from the haemolymph to the tubular lumen, of the catabolites and particularly of the uric acid (urate granules[26]).

As already shown in mesonephros and metanephros of the fowl[15] and in practically all excretory organs of vertebrates, both histochemically and biochemically[1,2,28,29], the CA plays a determining role in the excretion process and in urine acidification in uricotelic, ureotelic and ammoniotelic animals. Therefore, it can be assumed that the presence of the enzyme in the Malpighian tubules is a 'physiological necessity' and that its localization on surfaces is necessary for ionic transport and for preservation of the cellular pH[2].

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Immunocytochemical localization of cyclic AMP in *Tetrahymena*[1]

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Summary. Intracellular localization of adenosine 3',5'-cyclic monophosphate (cyclic AMP) in *Tetrahymena* was demonstrated immunocytochemically and changes in the staining pattern during culture growth and cell division were examined using an indirect immunofluorescent technique.

The presence of cyclic AMP, adenylate cyclase and cyclic AMP phosphodiesterase in *Tetrahymena* has been demonstrated biochemically, and changes in the levels of cyclic AMP were noted during culture growth and the cell cycle[3-6]. These studies suggested that cyclic AMP may possibly be a regulator in cell function. Intracellular localization of cyclic AMP in this organism has heretofore not been demonstrated, and we report here the immunocytochemical localization of cyclic AMP, and its changes during the culture growth and cell division in *Tetrahymena*. *T. pyriformis* NT-1 and *T. pyriformis* GL were grown at 39.5 and 28 °C, respectively, in enriched proteose-peptone medium, according to the method described by Thompson[7]. NT-1 cells were collected from the medium during the exponential and stationary phases and GL cells were harvested during the heat-synchronized cell division[6] by centrifugation at 500 × g. Pellets containing living cells were frozen immediately in an aluminium foil boat filled with OCT compound (Lab-Tek) by immersion in acetone and dry ice. Cryostat sections (10 μm) of unfixed samples were prepared and then airdried on glass slides. Localization of cyclic AMP was determined by an indirect immunofluorescent technique, as described by Steiner et al.[8]. Cyclic AMP-specific antiserum, prepared by the method of Steiner et al., was carefully screened for cross-reactivity with various other nucleotides and nucleosides using radioimmunoassay[9,10]. The cyclic AMP content in the cells was determined by the radioimmune method of Honma et al.[11].

Cyclic AMP fluorescence was localized in the cell membrane, cytoplasm, nuclear membrane, nucleus, cilia and

oral apparatus in *T. pyriformis* NT-1 (figure 1, a-c) and the specificity of the fluorescence was examined. This fluorescence was absent in cells when serum from a nonimmunized rabbit or phosphate buffered saline was used as the primary immunoreagent. In addition, the staining pattern obtained with antiserum to cyclic AMP was inhibited in a dose-dependent fashion when antiserum absorbed overnight with cyclic AMP was used as the primary immunoreagent. However, when other nucleotides or nucleosides were incubated with antiserum, the intensity of fluorescence did not significantly decrease. Affinity chromatography for antiserum adsorption was also performed, as described by Fallon et al.[12]. Passage of antiserum over an affinity column of Sepharose (Sigma) coupled to cyclic AMP eliminated the specific staining pattern which was obtained with the effluent antiserum. In all these examinations of fluorescence specificity, unspecific fluorescence was found only in the cytoplasmic granular structures (figure 1, d).

The histochemical evidence for the presence of cyclic AMP in the cilia and oral apparatus is in good agreement with reports suggesting the regulatory role of cyclic AMP in ciliary movement and regeneration[13,14]. The intensity of the fluorescence in cell membrane, cilia, oral apparatus and nucleus showed little change during culture growth, while that in the cytoplasm varied. Diffuse, intense cytoplasmic and bright perinuclear fluorescence was often observed in cells during the exponential phase (figure 1, b); on the other hand, the cells exhibited a weaker cytoplasmic fluorescence during the stationary phase as compared to cells during the exponential phase (figure 1, c). Cyclic AMP level in cells during the exponential phase was approximately 3 times that during the stationary phase (table); this agrees with an observation on another strain of *Tetrahymena*[3]. The change in cyclic AMP level appears to be greater than the change in cyclic AMP fluorescence, thereby suggesting that the increase in cyclic AMP during culture growth involves not only protein bound material but also free cyclic AMP, because a loss of free nucleotide from the tissue probably occurs during the staining procedure when unfixed frozen sections are used[8].

GL cells exhibited almost the same staining pattern as NT-1 cells, except for their weak nuclear membraneous fluorescence. Cytoplasmic fluorescence in the late stage of cell division markedly increased as compared with the fluorescence in the early stage of cell division (figure 2, a and b).

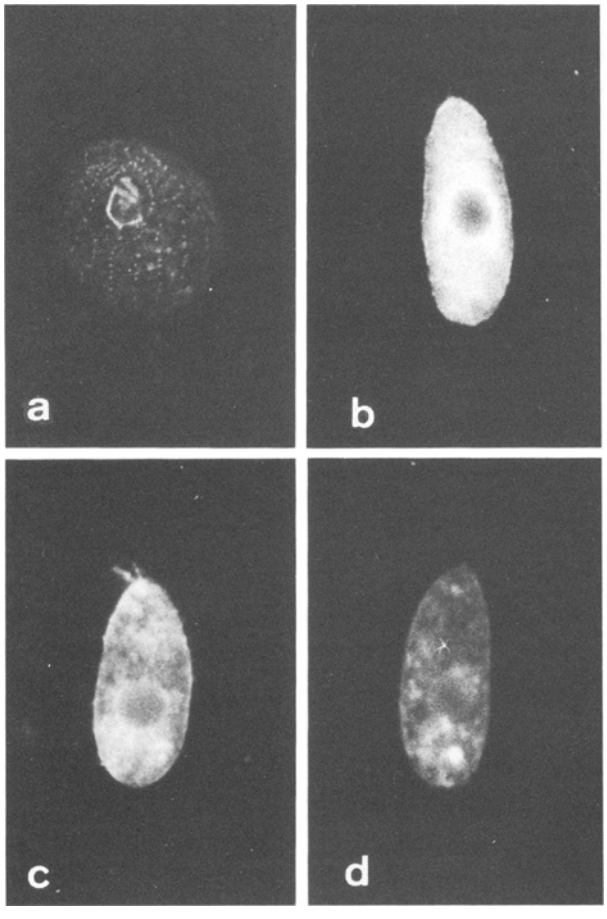

Fig. 1. Localization of cyclic AMP in *T. pyriformis* NT-1 and changes during the culture growth (× 120) for a, b, c, d. *a* Cyclic AMP fluorescences in the cortex structure of the anterior region of the *Tetrahymena* cell sectioned obliquely. Specific fluorescences were visible in the somatic ciliary rows, the membraneles and undulating membrane in the oral apparatus. *b* A longitudinal section of *Tetrahymena* cell during the exponential phase showing the diffuse specific cyclic AMP staining of cytoplasm and bright staining of the perinuclear area and nuclear membrane. *c* A longitudinal section of *Tetrahymena* cell during the stationary phase. Specific AMP fluorescences were visible in cell membrane, cilia and nuclear membrane. Note the weak cytoplasmic fluorescence. *d* A longitudinal section stained with antiserum absorbed with cyclic AMP (10^{-3} M). Unspecific fluorescence was observed only in the cytoplasmic granular structures.

Cyclic AMP levels during culture growth of *T. pyriformis* NT-1

	Time after the inoculation (h)	
	19 (exponential phase)	66 (stationary phase)
Cell count/ml	1.1×10^5	15.8×10^5
Cyclic AMP pmoles/10^6 cells	6.4 ± 0.7*	2.2 ± 0.3

The cyclic AMP levels represent the mean and SE of 6 samples. Cell counting was done using a Bürker-Türk hemocytometer. * Significantly different from the value seen in the stationary phase ($p < 0.05$).

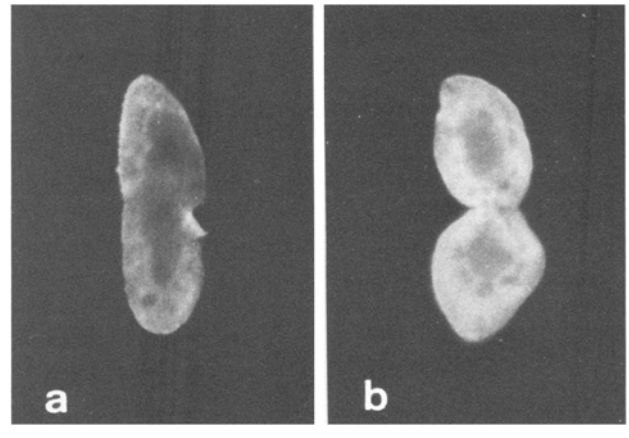

Fig. 2. Localization of cyclic AMP in *T. pyriformis* GL during cell division (× 160). *a* A cell in the early stage of cell division sampled at 60 min after the end of heat shock (EHS). Weak cyclic AMP fluorescence was seen in the cytoplasm. *b* Cell in the late stage of cell division sampled 75 min after the EHS. Markedly increased cytoplasmic fluorescence and weak intranuclear fluorescence were observed.

As this histochemical technique probably detects cyclic AMP bound to proteins and not the total cyclic AMP, as described above, we suggest that protein-bound cyclic AMP in the cytoplasm of *Tetrahymena* increases in the late stage of cell division. Although the role of cyclic AMP in this unicellular eukaryote is not well understood, our description of the intracellular localization of cyclic AMP may lead the way to eludication of the complex mechanisms involved.

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Angiogenic activity in the CSF in human malignancies

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Summary. Angiogenic activity, tested on the chorioallantoid membrane of the chicken embryo, was present in the CSF of patients with meningioma and glioblastoma and in patients with other malignancies with no clinical signs of CNS involvement.

From the pioneer study of Algire and Chalkey[3] a tumoral angiogenic factor has been widely recognized in human and animal[4-10] tumors, including brain tumors[11]. This factor was recently detected in the ocular fluid in cases of human eye tumors[12] using the vascularization of the chorioallantoid membrane of the chicken embryo. This study reports the results of a similar approach, studying the cerebrospinal fluid (CSF) of patients with primary brain tumors or other malignancies.

Materials and methods. CSF from 52 patients (9 with primary brain tumors, 17 with other malignancies in whom cerebral involvement was clinically excluded, and 26 controls) was aseptically obtained, filtered through a 0.22 µm Millipore filter, and stored at 4 °C after lyophilization of 0.2 ml aliquots; the tubes were given numbers to minimize subjective results. 32 of the patients were male and 20 female, and their ages ranged from 24 to 81 years (mean 57 years). The samples were implanted as powder in the chorioallantoid membranes of 9-day-old chicken embryos *(Gallus domesticus)*. The eggs were examined 48 h later under a stereomicroscope and photographs were taken. The membranes were subsequently fixed with saturated formalin solution and reevaluated.

Each sample was tested on 3 occasions and examined by more than 2 observers.

Results. The results are expressed in the table. The vascular responses were considered doubtful when the results were not uniform in the 3 tests performed in each case or there

	No cases	Vascular response		
		positive	doubtful	negative
Primary CNS tumors				
Neurinoma	2		2	
Meningioma	2	2		
Hemangioblastoma	1		1	
Papilloma	1			1
Astrocytoma	1		1	
Glioblastoma	2	2		
Other malignancies				
Carcinoma of the lung	7	3	4	
larynx	2		2	
tongue	2		2	
stomach	2		2	
prostate	1	1		
unknown origin	1			1
Leukemia	1	1		
Lymphoma	1	1		
Controls	26	1		25
Total	52	11	14	27