

derivative (VI), a compound believed to be a key intermediate in the transformation of cyclophosphamide into a cytotoxic species^{7,8}. Approaches to the synthesis of the 4-hydroxyderivative (VI) have included the attempted

reduction of 4-ketocyclophosphamide⁹. The availability of [³²P]4-ketocyclophosphamide, consequent upon the present studies, should facilitate the monitoring of such reactions.

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Zusammenfassung. Oxydation des (³²P) Cyclophosphamides durch KMnO₄ in Wasser oder Aceton führt zu 4-Ketocyclophosphamid und einem N-dechloroäthylierten Derivat des Cyclophosphamides, beides bekannte Metaboliten der Droge. Die Auftrennung der Produkte gelingt durch Dünnschichtchromatographie und die Richtigkeit der Strukturen wurde durch Massenspektrometrie nachgewiesen.

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Cyclic 3',5'-Adenosine Monophosphate in *Tetrahymena pyriformis*

Adenyl cyclase activity¹ and cyclic nucleotide phosphodiesterase² activity have been reported in the ciliate *Tetrahymena pyriformis*. It has been reported that theophylline increases glycogen level in *Tetrahymena*². Since methylxanthines are believed to affect the glycogen metabolism via cyclic AMP system³, it was of interest to study whether cyclic AMP⁴ is present in *Tetrahymena*.

Tetrahymena pyriformis, GL strain, is used in our studies. The cells are grown, harvested and washed as described earlier⁵. Finally, the cells are homogenized by freeze thawing 3 times in 1 ml 0.05 M sodium acetate buffer pH 6.2 plus 0.5 ml theophylline (30 mg/ml). The homogenate is centrifuged at 3000 rpm for 20 min and the supernatant taken for assay.

Radioimmunoassay method is used for the determination of cyclic AMP. The details of the method are well documented⁶. The radioimmunoassay kit is purchased from Schwarz/Mann. Protein is determined by the method of LOWRY et al.⁷ The Table includes the results. The amount of cyclic AMP present is expressed as picomoles per mg protein. As can be seen, cyclic AMP is present in cells grown in both media.

The intracellular concentration of cyclic AMP is extremely low, of the order of 10⁻⁶ M or lower, in most tissues. Cyclic AMP-mediated events are triggered by relatively small changes in the intracellular concentration of cyclic AMP. Hence, extremely sensitive methods are necessary to measure the intracellular levels of cyclic AMP. Among the various methods available, the sensitivity of the radioimmunoassay technique for cyclic AMP is reported to surpass that of the other methods⁶. In our experiments described here, we could detect the extremely

low level of the cyclic AMP in *Tetrahymena*, using the radioimmunoassay technique.

The significance of the presence of cyclic AMP in *Tetrahymena* can only be speculated at the moment. The effect of theophylline on glycogen metabolism in *Tetrahymena*, may now be examined by studying its effect on phosphodiesterase and the intracellular level of cyclic AMP.

It is reported that *Tetrahymena* contains catecholamines and serotonin and the growth of *Tetrahymena* is inhibited by a variety of adrenergic and/or serotonergic drugs². These data have earlier led to the hypothesis that *Tetrahymena* contains a primitive metabolic control system with several features in common with the intercellular metabolic systems found in metazoa². This similarity can now be extended further with our detection of cyclic AMP in *Tetrahymena* and the reported adenyl cyclase and phosphodiesterase activities. Work is now in progress to measure the effect of several drugs on the intracellular level of cyclic AMP in *Tetrahymena*.

Zusammenfassung. In *Tetrahymena pyriformis*, GL., wurde zyklisches AMP nachgewiesen.

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Determination of cyclic AMP in *Tetrahymena pyriformis*, GL.

Medium	Conditions	Amount of C-AMP (pmoles/mg protein)*
With glucose	25°C + shaking	2.70 ± 0.38
Without glucose	25°C no shaking	1.07 ± 0.38

* The assay is carried out with different aliquots of the homogenate (25 to 100 µl) in duplicate.

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⁴ Abbreviations used: cyclic AMP - cyclic 3',5'-adenosine monophosphate.

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