the method of REED et al.[7] using 1-^{14}C-acetylcholine (New England Nuclear) as a substrate.

For measurement of growth retardant activity, mung bean seeds were soaked overnight in running tap water and planted in petri dishes on Whatman No. 1 paper wetted with 5 ml of test solution and adding 2 ml of the same solution on the 3rd day. The seedlings were grown under continuous light at 25°C. The number of secondary roots was determined on the 6th day.

The growth retardants used were: tributyl-2,4-dichlorobenzylphosphonium chloride (Phosfon-D); 2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidine carboxylate methyl chloride (AMO-1618); (2-chloroethyl) trimethylammonium chloride (CCC); 1-*p*-mentanol, 2-dimethylamino-4-bromobenzyl bromide (Q76) and 1-*p*-mentanol, 2-dimethylamino-2,5-dimethylbenzyl chloride (Q80).

Results and discussion. The properties of the bean ChE are summarized in Table I. The data in this table represent only one ChE, as confirmed by the similarity in the effects of a number of inhibitors on the rates of hydrolysis of several choline esters by the enzymatic preparations used. Table I shows that the bean enzyme is not identical with any animal ChE. However, it is similar in some important properties to AChE. Although the physiological role of this enzyme is not yet clear and there is no evidence that ACh is the natural substrate of the enzyme, preliminary observations (M. J. JAFFE, unpublished) indicate that it probably functions as AChE to regulate the content of ACh in the root system.

NEWHALL[8,9] reported an excellent positive correlation between the ability of quaternary ammonium derivatives of (+)-limonene to inhibit human serum-ChE and their ability to retard the growth of various plant seedlings. Following his observations, we studied the effects of recognized plant growth retardants on the activity of bean ChE in comparison to their retardation of the production of secondary bean roots. Table II shows that there was a significant positive correlation between the two effects studied; the more effective a compound was at retarding root production, the more it inhibited the enzyme. At present, the meaning of these observations is still obscure, but two possible explanations may be offered: 1. Endogenous ACh may be a native growth retardant and inhibition of the ChE allows its accumulation in the root system. 2. The enzyme or enzymes which are responsible for root growth are biochemically similar to the ChE and the ChE is acting as a representative model when in vitro. More research is needed to clarify the observations reported[10,11].

Table II. Effect of plant growth retardants on the in vitro activity of bean root ChE and on the production of secondary roots of mung bean seedlings

Growth retardant	I_{50}[a] (*M*)	
	ChE activity[b]	Secondary root production[c]
Phosfon-D	3.3×10^{-5}	2.0×10^{-5}
Q80	2.3×10^{-4}	6.6×10^{-4}
AMO-1618	2.1×10^{-4}	7.6×10^{-4}
Q76	3.5×10^{-3}	1.2×10^{-3}
CCC	9.0×10^{-2}	4.3×10^{-2}
Coefficient of correlation (r)		$+0.99$[d]

[a] I_{50} = 50% inhibition. [b] ChE activity was assayed by the method of ELLMAN et al.[6], using acetylthiocholine as a substrate. [c] Each datum of root production represents the average of 5 replicates, 10 plants per replicate. [d] $P < 0.01$.

Résumé. Une nouvelle cholinestérase des racines des haricots a été isolée et partiellement purifiée. L'affinité de la cholinestérase des haricots pour l'acétylcholine est comparable à celle de l'acétylcholinestérase animale, mais elle diffère de cette dernière par sa réaction aux inhibiteurs et par d'autres propriétés. Une corrélation positive entre l'inhibition de la production des racines des haricots par plusieurs retardants de la croissance végétale et leurs effets inhibitoires sur la cholinestérase a été établie dans cette étude.

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[11] We wish to thank te Mobil Chemical Corp. for a gift of Phosfon-D, and Dr. W. F. NEWHALL of a gift of the compounds Q76 and Q80.
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Adrenergic Mechanism in *Tetrahymena*. I. Changes in Monoamine Oxidase Activity During Growth

JANAKIDEVI et al.[1,2] first found biogenic amine, such as noradrenaline, adrenaline and serotonin, in the ciliated protozoan, *Tetrahymena pyriformis* W. Recently, we reported that this protozoan possesses monoamine oxidase (MAO) and diamine oxidase[3]. It was suggested previously[1-6] that catecholamine (CA) may participate in metabolism in *Tetrahymena* as it does in mammals, though its precise role in the growth of the protozoan is still unknown. This paper reports the relationship between MAO activity and cell proliferation and the influence of adrenaline on enzyme activity.

Materials and methods. Tetrahymena pyriformis W, was cultured in a medium containing 2% polypepton and 0.1% yeast extract at pH 7.2, 26°C. After growth for 96 h in this medium, cells were inoculated into fresh medium and the growth rate was determined turbidimetrically, as described previously[6]. Synchronization was carried out by the heat shock method of SCHERBAUM and ZEUTHEN[7], with a slight modification: cells were subjected to 8 heat shocks, each of 30 min heat shocks at 32°C, separated by intervals of 30 min at 26°C. The transfer to inorganic medium (50 m*M* NaCl, 1 m*M* $MgSO_4 \cdot 7H_2O$ and 10 m*M* KH_2PO_4) was done by centrifugation prior to the 7th shock by washing the cells 3 times in inorganic medium. The synchronized cultures were then incubated with and without L-adrenaline (5×10^{-5} *M*) at 26°C, and samples were removed from the medium by pipette every 15 min for 2 h. The procedures used for enzyme preparation and

assay of activity were as reported previously[3]. Enzyme activity was expressed in terms of the amount of ammonia liberated from noradrenaline.

Results. The growth curve and time course of change in MAO activity in growing cultures are shown in Figure 1. The enzyme activity decreased in the exponential growth phase and increased in the stationary phase. The changes in activity in synchronized cells cultured with and without adrenaline after the end of heat treatment (EHT), are illustrated in Figure 2. Cytokinesis was observed by microscopy to begin about 60 min after EHT under these conditions, and in control cells enzyme activity decreased at this time. After cell division, the activity increased again and remained high until about 90 min after EHT. Ammonia production decreased again from 90 min after EHT, at the time when DNA synthesis was initiated in this system[8]. On the other hand, addition of adrenaline resulted in immediate increase in MAO activity and then its decrease.

Discussion. As to the relation between cell proliferation and MAO activity, SOUTHGATE et al.[9] showed that the activity in human endometrium was low during the non-secretory and early secretory phases but showed a rapid increase at the beginning of the late secretory phase. Later they[10] reported that MAO activity in rat uterus was higher after treatment with progesterone than after treatment with estradiol. The present results on MAO activity in *Tetrahymena* are quite different from that obtained in bacteria. YAMADA et al.[11] reported that bacterial MAO activity increased during the initial stage of growth and then decreased semilogarithmically. These findings indicate that the significance of MAO for growth of *Tetrahymena* differs from that for growth of bacteria.

Previously, BLUM et al.[12] reported that the CA content of *Tetrahymena* differed from that found by JANAKIDEVI et al.[1]. This contradiction can be explained from our results, which show that the CA content varies with the growth phase.

In synchronized cultures of *Tetrahymena*, MAO activity was found to decrease during cell division and DNA replication phase. The periodical change in MAO activity in cells treated with adrenaline differs from the change in control cells. These results are probably related to our recent observation[8] that adrenaline inhibited protein and/or RNA synthesis in the late G_1 and G_2 phases.

BULLOUGH et al.[13] demonstrated that adrenaline inhibited the mitosis in mouse epidermis. They[14] suggested that the adrenaline-chalone complex affects a stage before cell division and inhibits cytokinesis in mouse epidermal cells.

On the other hand, MAVRIDES et al.[15] showed that the activity of tyrosine aminotransferase is higher in *Tetrahymena* cells in the stationary phase than in the exponential phase. The precise relation of their data with ours is still obscure. These findings suggest that some adrenergic mechanism in *Tetrahymena* is involved in cell proliferation.

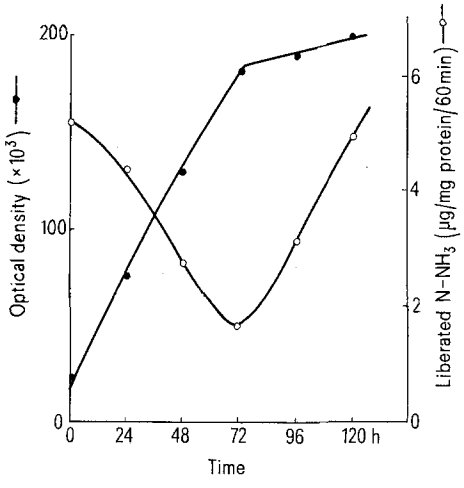

Fig. 1. Monoamine oxidase activity during growth of cultures of *Tetrahymena*.

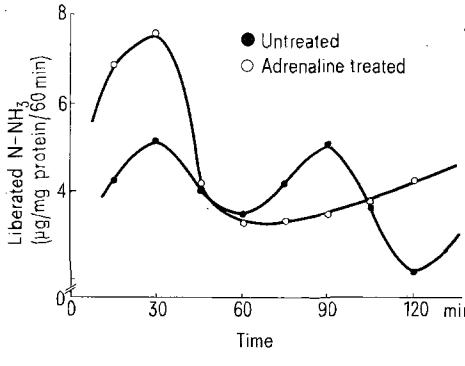

Fig. 2. Effect of adrenaline on monoamine oxidase activity in synchronized cultures of *Tetrahymena*.

Zusammenfassung. Es wird festgestellt, dass die Aktivität der Monoaminooxidase bei Ciliaten *Tetrahymena pyriformis* in der exponentialen Wachstumphase abnimmt und in der stationären Phase zunimmt. In der synchronisierten Zellpopulation verringert sich die Enzymaktivität in beiden Phasen der Zellteilung und der DNS-Synthese.

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