The final acidity was pH 3, but when medium (d) was used, including a buffer, no increase in ammonium ion uptake occurred, did not significantly affect the free amino acid pool and no reduction was detected in the extracellular amino acids found in the medium. It appears as if this species at least can withstand low pH values without marked interference with its metabolism. When the percentage composition of the free amino acid pools (Table I) are compared they are found to only partly agree with BENT and MORTON[9] in that they change in size but not greatly in composition in response to downward pH drift. There is no obvious relationship between the free pools of *Clavariopsis*, *Penicillium*[7], and *Mucor*[11], but there may be a relationship between the high concentration of alanine and lysine in *Clavariopsis* and *Heliscus submersus*[4] (both are aquatic Hyphomycetes) though CLOSE[12] suggests little hope of classification of fungi by free amino acid pool composition.

The proportions of protein amino acids (Table II) to free amino acids shows little relationship, especially aspartic acid which frequently occurs in low concentrations in the free pool but high in protein whilst the reverse is frequently true of alanine. This is in agreement with results for yeasts[13] *Penicillium*[7] and *Mucor*[11].

Table III. Amino acid composition of bulk protein of *Clavariopsis aquatica* compared with 2 organisms of GC 25%

	Mycoplasma[a] *mycoides*	*Clavariopsis*[b] *aquatica*	*Tetrahymena*[c] *pyriformis*
% Guanine-cytosine content of DNA	24.8	?	25.0
Stable amino acids[d]	Percentage amino acid composition		
Arginine	4.0	3.8	6.1
Lysine	14.3	13.6	11.9
Histidine	2.8	0.3	2.8
Glutamate	15.7	14.1	17.6
Aspartate	16.7	16.2	16.5
Alanine	9.9	10.3	10.3
Proline	5.4	2.6	5.1
Valine	7.2	8.1	8.9
Tyrosine	4.6	1.5	4.3
Phenylalanine	5.6	6.3	6.0
Leucine	12.1	11.6	11.7

[a] CHELTON et al.[15]. [b] Results for medium b. [c] SUEOKA[14]. [d] For explanation of this term see CHELTON et al.[15].

2. Protein amino acids. Table II shows, with few exceptions, good agreement between all percentage values of amino acids of bulk protein of *Clavariopsis* regardless of nitrogen source supplied. This is in agreement with the findings of BENT and MORTON[7] for fungi and SUEOKA[14] for various bacteria.

SUEOKA[14] has shown a relationship between percentage composition of guanine-cytosine (%GC) in DNA and amino acid composition of bulk protein for bacteria. This was further verified by CHELTON et al.[15]. According to SUEOKA[14], a positive correlation exists between %GC and % alanine, arginine and proline and an inverse correlation between % lysine, aspartate, glutamate, tyrosine and phenylalanine. If the results of SUEOKA[14] and CHELTON et al.[15] are compared with *Clavariopsis* (Table III) it corresponds closely to organisms with a %GC of 25, e.g. *Mycoplasma mycoides* and *Tetrahymena pyriformis*. The exceptions are tyrosine and proline.

In *Penicillium griseofulvum*[7] arginine, alanine and proline correspond to a GC ratio of 25%, but lysine, aspartate, tyrosine and luecine correspond to GC 70%. It would clearly be of interest to investigate %GC for *Clavariopsis* and many more fungi, particularly *Penicillium griseofulvum*[7], to determine the possibility of applying SUEOKA's[14] suggestions to fungi.

Résumé. On a trouvé que *Clavariopsis aquatica* tolère une grande acidité. Le pourcentage des amino-acides qui composent la protéine fongique a été très peu affecté par la source d'azote et n'a aucun rapport avec la composition de la masse commune des amino-acides libres. La pourcentage des amino-acides qui composent la protéine correspond à un pourcentage de guanine/cytosine de 25.

D. R. THORNTON and M. GRIFFIN

Department of Biology, University of Salford, Salford M5 4WT, Lancs. (England), 23 September 1970.

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Amine Oxidative System in *Tetrahymena pyriformis* W

In 1966, JANAKIDEVI et al.[1] found that the ciliated protozoan, *Tetrahymena pyriformis* W, possesses noradrenaline (NA) and adrenaline (A), while the flagellated protozoan, *Crithidia fasciculata*, has only NA. Further, in these protozoa, isotopically labelled precursors were incorporated into these catecholamines as in mammals. They also suggested that the decomposition of NA was due to monoamine oxidase in *C. fasciculata*. Based on these findings, BLUM[2] studied the effect of reserpine on *Tetrahymena* and found that it inhibited the growth and decreased the catecholamine content.

Previously, we reported that low concentration of α-adrenergic blocking agents promoted the growth of *Tetrahymena*, while β-adrenergic blockers were inhibitory[3,4]. Thus it was of interest to investigate the nature of the enzyme involved in catecholamine metabolism in *Tetrahymena*. This paper is on the presence and nature of an oxidative deamination system for catecholamine in *T. pyriformis*.

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Materials and methods. *T. pyriformis* W was cultured in medium containing 2% polypepton and 0.1% yeast extract at pH 7.2, at 26 °C, for 96 h. The cells were harvested by centrifugation at 700*g* for 5 min and washed with phosphate buffer (0.2*M*, pH 7.2). After washing, the pellet was suspended in phosphate buffer and kept at −22 °C until assays were made. The enzyme was prepared by the freezing-thawing method[5]. Protein determination was performed by the method of LOWRY et al.[6].

The reaction mixture, composed of enzyme preparation (0.48–0.53 mg protein/ml), 1 m*M* of substrate and phosphate buffer in a total volume of 5.0 ml, was incubated for 60 min at 26 °C. The reaction was stopped by the addition of 10*N* H_2SO_4. These mixtures were centrifuged at 1500*g* for 5 min and the supernatant was used for the measurements. The ammonia produced was determined by a modification of the microdiffusion method[7].

Results. As shown in Figure 1, linear relationships were observed between enzyme activity and the concentration of protein or NA. Similar results were obtained using tyramine and/or benzylamine as substrate. In these experiments, enzyme activity was measured as disappearance of the substrate, tyramine[8], or by production of the metabolite, benzaldehyde, for benzylamine[9] (data not shown).

The effect of pH on enzyme activity was determined with NA as substrate and activity was estimated as the amount of ammonia produced. As shown in Figure 2, the optimal pH for enzyme activity was 7.4.

Table I. Substrate specificity of amine oxidase in *Tetrahymena*

Substrate (1 m*M*)	N–NH_3 produced (µg/mg protein/ 60 min)	Relative activity (%)
Noradrenaline	4.49	100
Normetanephrine	3.03	67
Tyramine	2.02	45
5-hydroxytryptamine	1.57	35
Benzylamine	1.12	25
Histamine	0.89	20

Table II. Effect of inhibitors on amine oxidase in *Tetrahymena*

Inhibitor (1 m*M*)	Inhibition (%) Substrate (1 m*M*)	
	Noradrenaline	Histamine
Nardil	24	0
Catron	20	0
Iproniazide	16	0
Ephedrine	28	0
KCN	0	100
Hydroxylamine	0	100

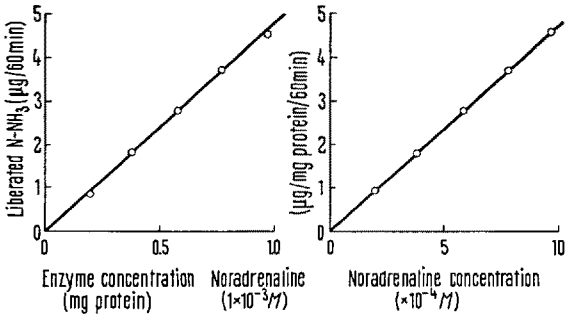

Fig. 1. Effect of enzyme and substrate concentrations on the liberation of ammonia from noradrenaline by the amine oxidase of *Tetrahymena*.

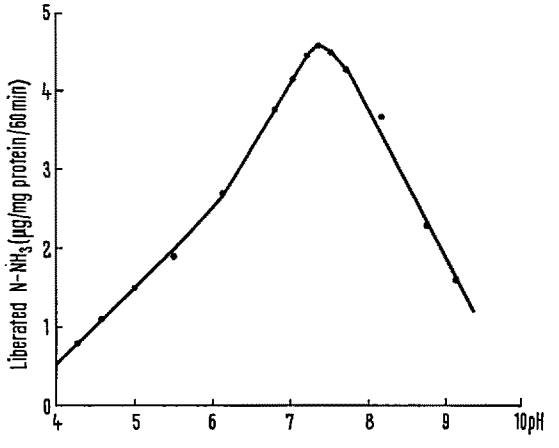

Fig. 2. Effect of pH on enzyme activity with noradrenaline as substrate. Buffers: acetate (pH 4–6), phosphate (pH 6–8) and *Tris*-HCl buffer (pH 8–9).

Substrate specificity was determined estimating activity as ammonia liberation and the activities with various substrates were expressed as amounts of N–NH_3 liberated and as activities as percentage of that with NA. The results are listed in Table I. For comparison, histamine was also tested as substrate. Table I shows that most ammonia was liberated with NA as substrate.

Table II shows the effects of monoamine and diamine oxidase inhibitors on the liberation of ammonia from NA and histamine. The compounds, Nardil, Catron, iproniazide and ephedrine, which inhibit monoamine oxidase in mammals, interferred with ammonia production from NA in *Tetrahymena*. However, diamine oxidase inhibitors, such as potassium cyanide and hydroxylamine, did not affect enzyme activity. On the other hand, ammonia liberation from histamine was not inhibited by diamine oxidase inhibitors.

Discussion. The first report of a metabolic pathway for monoamine in micro-organisms was that of JANAKIDEVI et al.[1]. In other micro-organisms, YAMADA et al.[10] using *Aspergillus niger* cultured in medium containing monoamines, found that maximal formation of amine oxidase occurred during the initial stage of growth. Then the enzyme disappeared semilogarithmically. No enzyme

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formation was observed on addition of ammonia, nitrate, urea or amino acid. They suggested that the amine oxidase might be adaptive and that it was responsible for growth of the fungus with amines as the sole nitrogen source. Namely, as described by others also[11-13], the amine oxidase was only found in some micro-organisms which were cultured in medium with amine. These results show that the enzyme is genotypic in these organisms and that enzyme synthesis begins in adaptation to amines. However, it is unknown whether these organisms have biologically active amines.

In *T. pyriformis*, the optimal pH of the enzyme was 7.4 with NA as substrate. This value is similar to that of the monoamine oxidase of mammals. Unlike the mammalian enzyme[14], NA and normetanephrine were better substrates for the enzyme of *Tetrahymena* than tyramine. Recently, YOUDIM et al.[15] observed that multiple forms of monoamine oxidase exist in rat brain, differing in pH optima and substrate specificities. Therefore, it is uncertain whether the difference between the enzyme in *Tetrahymena* and in mammals is a phylum difference. Like the mammalian enzyme, with monoamine as substrate, deamination by *Tetrahymena* enzyme was depressed by monoamine oxidase inhibitors but not by diamine oxidase inhibitors, while the reverse was found with histamine as substrate.

These results suggest that *T. pyriformis* has both monoamine- and diamine- oxidase. When *Tetrahymena* was cultured in medium without amine, the enzymes were expressed as phenotypes. Thus the significance of amine oxidase differs completely in *T. pyriformis* and *C. fasciculata*, from in other micro-organisms. In this connection, it should be mentioned that we have recently observed that monoamine oxidase activity is closely related to the growth conditions or the cell cycle of *Tetrahymena*. Results will be published elsewhere.

Zusammenfassung. Nachweis einer Aminooxydase-Aktivität beim Ciliaten *Tetrahymena pyriformis*, die derjenigen der Säugetiere ähnlich ist.

H. IWATA, K. KARIYA and H. OKAMOTO[16]

Department of Pharmacology, Faculty of Pharmaceutical Sciences, Osaka University, 5, Toneyama, Toyonaka, Osaka-fu (Japan),
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Frontal Ganglion and Water Balance in *Periplaneta americana* L.

CLARKE and LANGLEY[1,2] observed that the removal of the frontal ganglion in third-instar larvae of locusts 'results in an immediate cessation of growth, no matter at what time during an instar the operation is performed'. The weight of the operated animals remains constant until they die. The authors concluded that the failure of growth is due to a general failure of protein synthesis following frontal ganglionectomy. The ganglion is supposed to be a relay centre for the transmission of nervous impulses from stretch receptors of the pharynx to the neurosecretory system in the brain, which controls the protein synthesis. Interruption of this pathway results in a cessation of the production and/or release of neurosecretory material in the brain[3,4]. In contrast to CLARKE et al., HIGHNAM[5] considers the inhibition of growth which follows the removal of the frontal ganglion to be the result of 'semi-starvation'.

In our experiments with larvae of the cockroach *Periplaneta americana*, we found quite normal regeneration of the metathoracic legs after ganglionectomy[6]. We therefore conclude that the protein synthesis cannot be blocked in animals without the frontal ganglion. Ganglionectomized larvae die within some days, if they get neither food nor water. They lose considerably in weight. The decrease in body weight occurs the more slowly and the animals survive the longer, the higher the relative humidity of the surroundings. The loss of body weight during 24 h in starving ganglionectomized animals at a relative humidity of about 0% corresponds to the loss in dead animals under the same conditions. With sham-operated animals the loss in weight is significantly lower (Table I).

The normal animals, in contrast with ganglionectomized or dead animals, are obviously able to reduce their loss in body weight. The decrease in body weight corresponds to an increase in osmolarity of the hemolymph. Larvae of about the same size were held under the same contions and examined 6 days after their last molt (Table II). The cutting of only one of the frontal connectives has no significant effect upon the freezing point of the hemolymph. In contrast with this, already 2 days after cutting

Table I. Loss in body weight in % of the initial weight during 24 h in an exsiccator above $CaCl_2$

	No. of observations	Mean value of the loss in body weight $\pm s_{\bar{x}}$	Level of significance
Sham-operated	10	6.6% ± 0.50	$p < 0.01$
Ganglionectomized	12	11.4% ± 0.75	$p < 0.02$
Dead	16	10.9% ± 1.23	$p > 0.5$

Temperature 28 ± 0.5 °C.

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