

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 also¹¹⁻¹³, 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 enzyme¹⁴, NA and normetanephrine were better substrates for the enzyme of *Tetrahymena* than tyramine. Recently, YODIM 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.

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¹¹ U. BACHRACH, J. biol. Chem. 237, 3443 (1962).

¹² Y. MAKI, Y. ITSUNO, M. TAKESHITA, S. MIYATA and S. TANAKA, Kumamoto med. J. 17, 90 (1967).

¹³ H. YAMADA, H. UWAJIMA, H. KUMAGAI, M. WATANABE and K. OGAWA, Agric. Biol. Chem. 31, 890 (1967).

¹⁴ W. NORMAN, Arch. Biochem. Biophys. 91, 182 (1960).

¹⁵ M. B. H. YODIM, G. G. S. COLLINS and M. SANDLER, Nature, Lond. 223, 626 (1969).

¹⁶ We wish to thank Miss K. TANAKA and Miss Y. NANBA for their help in this experiment.

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., HIGHNAM⁵ 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 ganglionectomy⁶. 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 conditions 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 CaCl₂

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

Temperature $28 \pm 0.5^\circ\text{C}$.

¹ K. U. CLARKE and P. A. LANGLEY, J. Insect Physiol. 9, 363 (1963).

² K. U. CLARKE and P. A. LANGLEY, J. Insect Physiol. 9, 411 (1963).

³ C. GILLOT, Helgoländer wiss. Meeresunters. 9, 141 (1964).

⁴ K. U. CLARKE and C. GILLOT, Nature, Lond. 208, 808 (1965).

⁵ L. HILL, W. MORDUE and K. HIGHNAM, J. Insect Physiol. 12, 1197 (1966).

⁶ H. PENZLIN, J. Insect Physiol., in press (1970).

Table II. Osmolarity (depression of the freezing point Δ °C) in the hemolymph of larvae of about the same size 6 days after the last molt

	No. of observations	Mean value of Δ °C \pm $s_{\bar{x}}$	Level of significance
Normal animals	35	0.66 ± 0.014	$\left. \begin{array}{l} p > 0.05 \\ p < 0.001 \\ p < 0.001 \\ p < 0.01 \end{array} \right\} p < 0.001$
One frontal connective cut (2 days before)	21	0.60 ± 0.033	
Both frontal connectives cut (2 days before)	35	0.75 ± 0.017	
Both frontal connectives cut (3 days before)	12	0.86 ± 0.043	

Temperature 28 ± 0.5 °C; about 100% RH.

both of the frontal connectives a significantly ($p < 0.001$) higher depression of the freezing point than in normal animals is to be observed. This operation has the same effect as the removal of the entire frontal ganglion. 3 days after cutting both the frontal connectives, the depression of the freezing point continued to increase. It is significantly higher than that after 2 days ($p < 0.01$). The difference in osmotic pressure of the hemolymph between normal animals and those with cut frontal connectives days after operation amounts to 2.4 atmosphere.

The increasing depression of the freezing point corresponds to a decrease in hemolymph volume. 3 days after cutting both the frontal connectives, it is already difficult to get samples of blood; later on it is quite impossible: no blood can be withdrawn from the fresh wounds.

These results show that starving animals lose much water when the frontal ganglion is removed or both the frontal connectives are cut. The loss in body weight is as large as with dead larvae under the same conditions. The animals with intact connection between the frontal ganglion and the tritocerebrum can reduce this water loss. A diuretic factor is located in the neurosecretory centres of the pars intercerebralis⁷⁻⁹. It is possible that the frontal ganglion receiving impulses from osmorecep-

tors exerts an inhibitory influence upon these neurosecretory centres. Cutting the connectives results in a loss of inhibition and consequently in an increasing release of the diuretic factor from the brain.

Zusammenfassung. Der Gewichtsverlust bei frontalganglionektomierten Schaben innerhalb von 24 h im Exsikkator ist wesentlich grösser als derjenige bei scheinoperierten Tieren. Er erreicht Werte, wie sie bei toten Tieren auftreten. Der Gewichtsverlust ist mit einer signifikanten Zunahme der Gefrierpunkterniedrigung in der Hämolymphe verbunden. Durchtrennung beider Frontalkonnective hat dieselbe Wirkung wie Ganglionektomie.

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⁷ M. J. BERRIDGE, *J. exp. Biol.* 44, 553 (1966).

⁸ K. C. HIGHNAM, L. HILL and J. GINGELL, *J. Zool., Lond.* 147, 201 (1965).

⁹ W. MORDUE, *J. Insect Physiol.* 15, 273 (1969).

The Resistance of in situ Perfused Lymph Trunks and Lymph Nodes to Flow

During active or passive movements of a hindlimb, the pressure in its lymphatics increases¹. When the flow of lymph is substantially increased by plasmapheresis, perinodal oedema develops². The two phenomena presumably have a common cause: obstruction to the lymph flow by the lymph nodes. To see whether the nodes really obstruct the flow, we studied the relation obtaining between flow and pressure before and behind the nodes in the lymphatic system.

Dogs of either sex, weighing 14–20 kg, were anaesthetized with chloralose. In a group of 20 dogs the thoracic duct through the intestinal lymphatic trunk, and in one of 27 animals the lymph nodes in various body regions, through their afferent lymph vessels, were perfused in situ. The polyethylene cannula for perfusion of the thoracic duct was inserted through a 5 cm long central stump of the intestinal lymphatic trunk. For recording pressure, a lateral branch of the intestinal lymph trunk and the central stump of the left cervical lymphatic trunk were cannulated. A fine double-barrelled polyethylene cannula was introduced into an afferent lymphatic to within 5–10 mm of the lymph node; one barrel was used for perfusion, the other for pressure recording. A Schwarzer transducer and polygraph (Physioscript)

were used. We perfused the thoracic duct with a 1:1 mixture of dog plasma and physiological saline (Harvard Peristaltic Pump: Model 500–1200), and the lymph nodes with physiological saline alone (Harvard Compact Infusion Pump: Model No. 975). The perfusion fluid was stained with Evans' blue to detect possible ruptures. Perfusion was started with 2.5 ml/min for lymph trunks, and mostly 0.005 ml/min for lymph nodes, and doubled every 2 min. Thoracic duct perfusion reached values occasionally exceeding 20 ml/min, and that of the lymph node 0.1–0.3 ml/min.

In the mathematical treatment of flow (F) and pressure (P) we proceeded from the equation $P = b(F)^a$ where F = perfusion rate and P = pressure increment (belonging to F) above baseline for thoracic duct perfusion, or F = perfusion rate less F_1 and P = pressure less P_1 for lymph node perfusion; P_1 is the pressure belonging to the lowest value of perfusion rate F_1 .

¹ C. K. DRINKER and M. E. FIELD, *Lymphatics, Lymph and Tissue Fluid* (Williams and Wilkins Co., Baltimore 1933).

² M. PAPP, Postdoctoral theses, Budapest 1970.