$(Na^+ + K^+)$-ATPase stimulation (%) and % desmosterol of total sterols of erythrocyte ghosts ($n = 8$)

	A		B	C		D
Stimulation of Mg^{++}-ATPase by Na^+ and K^+ (%)	113.1 (87.7–149.5)		92.3 (64.6–118.8)	50.2 (44.9–54.1)		44.5 (39.5–49.1)
P		< 0.005			< 0.001	
Desmosterol of total sterols (%)	75.4 (57–90)		61.6 (43–75)	12.8 (11–15)		— —
P		< 0.005				

For the meaning of ABC and D see legend of Figure 1. P was determined by using the paired Student t-test.

And the maintenance of a fluid-like environment due to its lipid constituents is the prerequisite for an optimal function of transport enzymes, as could be shown by spin label studies[13,14].

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Summary. Cholesterol of red blood cells (RBC) is readily exchanged by desmosterol and vice versa. The resulting alteration in the sterol composition influences the specific $(Na^+ + K^+)$-ATPase activity. It is suggested that this effect is due to an altered membrane fluidity.

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Monoamine Oxidase Activity in *Helix pomatia*

It has been reported that although monoamine oxidase (MAO) exists in molluscan nervous tissue, it is not able to metabolize a tryptamine or serotonin substrate[1]. However, a number of other experiments[2–7] have shown both 5-hydroxytryptamine (5-HT) and dopamine (DA) to be slightly metabolized by presumably MAO, though the activity is very low. It was therefore decided to analyze the distribution and nature of MAO in the snail *Helix pomatia* in order to establish the functional significance of the enzyme in the nervous system.

Methods and materials. Tissues were dissected from active snails into ice cold snail saline[8], weighed and then homogenized in ice cold 0.15 *M* KCl, giving 10–100 mg tissue/ml. Aliquots were taken for protein assay[9]. MAO activity was analyzed according to Wurtman and Axelrod[10] with minor variations. The reaction mixture had a total volume of 60 µl consisting of 20 µl homogenate, 20 µl phosphate buffer (pH 7.4, 0.5 *M*), and 20 µl ^{14}C-tryptamine solution (47 mCi/mmole; 0.05 µCi diluted with unlabelled tryptamine to give 12 nmole/20 µl in 0.01 *N* HCl). The mixture was incubated at 37 °C for 60 min, stopped by the addition of 70 µl 2 *N* HCl and 100 µl therefrom twice extracted with 2 ml toluene. The toluene was separated from the aqueous phase by centrifugation, added to 10 ml scintillation fluid and counted in a Packard Liquid Scintillation Counter. 2 to 3 determinations were made from each tissue homogenate. Blank values were obtained as zero-time reactions. The activity was corrected for the efficiency of the extraction procedure and expressed as nmole tryptamine metabolized/g tissue/min and nmole tryptamine metabolized/g protein/min.

In several cases, ^{3}H-5-HT (17.3 Ci/mmole, 0.2 µCi diluted with unlabelled 5-HT to give 6 nmole/20 µl):, ^{3}H-DA (7.2 Ci/mmole, 0.1 µCi diluted to give 6 nmole/20 µl) or ^{14}C-tryptamine (47 mCi/mmole, 0.1 µCi diluted to give 6 nmole/20 µl) was used as substrate and the resulting products extracted into 2 ml of ethylacetate/benzene (1:1 by volume).

To test drug effects, dissected suboesophageal ganglia were partially desheated and incubated for 60 min at 20 °C in snail saline containing various concentrations of drugs together with 0.1 mg/ml ascorbic acid. The ganglia were then blotted dry and their MAO activity was analyzed.

Results and discussion. A great variation of MAO activity occurred in the different tissues (see Table), being highest in the liver (62.11 nmole/g protein/min) and absent in the albumen gland, flagella and radula retractor muscle. In the nervous tissue, the MAO activity (nmole/g protein/min) in the buccal ganglion was 26.81, while in the supraoesophageal and the suboesophageal ganglia it was 16.86 and 13.34 respectively. This is less than 1% of that reported for vertebrate nervous tissue[11], but is similar to that found in other molluscs[12].

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MAO activity proved to be linear for a minimum of 60 min at temperatures between 20 °C–37 °C. The Q_{10} (27 °C–37 °C) was found to be 1.9. At 0 °C no activity was found, which is interesting in view of the fact that snails can survive for months at this temperature. This, together with other data[13], showing an uptake mechanism for 5-HT to exist at 0 °C, supports the idea that MAO plays only a minor role in the inactivation of neurotransmitters in the snail.

MAO properties were studied in more detail in the suboesophageal ganglia. The K_m value for tryptamine was 2.1×10^{-5} M, similar to that found in the rabbit brain of 1.6×10^{-5} M[11]. Using 5-HT and DA as substrates, rather than tryptamine, it was found that 5-HT was deaminated at a rate of only 20% and DA only 50% of that of tryptamine. This contrasts with studies in the vertebrates where higher specific activities were found for 5-HT and DA substrates in contrast to tryptamine[11].

The effects of various drugs on MAO activity were also studied. Nialamide (10^{-4} M), a MAO inhibitor, produced a 50% decrease of MAO activity. Chlorpromazine (10^{-4} M), a DA receptor blocker, also inhibited MAO by 50%: Concentrations of less than 5×10^{-5} M of either drug had no measurable effect. Reserpine (5×10^{-5} M or greater) enhanced the MAO activity, with a 50% increase at 10^{-4} M. Incubation of ganglia with 5-HT (10^{-5} $M - 10^{-3}$ M) resulted in a non-linear decrease in measured activity, though this might be due to competition of the accumulated 5-HT with the ^{14}C-tryptamine.

The effects of pargyline (MAO inhibitor) were also examined. 5-HT deamination was only inhibited with pargyline concentrations greater than 5×10^{-4} M. DA and tryptamine deamination were both partially inhibited by pargyline between 5×10^{-4} M and 5×10^{-5} M. Little effect was seen at concentrations lower than 10^{-6} M. This is interesting in view of the fact that multiple forms of MAO have been proposed[14–16] to occur in the vertebrates, based on the differential degradation of various monoamines and the actual separation of 'isoenzymes' from purified preparations. The suggestion has been put forward that pargyline (MAO inhibitor) selectively inhibits the B type enzyme[17] (substrate tyramine) at low concentrations, while the A type enzyme (where 5-HT is a substrate) is only inhibited by higher concentrations of pargyline. DA und tryptamine (being) substrates for both types, are partially inhibited at low concentrations. Such is found to be the case in the snail. It is therefore possible that more than one form of MAO occurs in the snail, though the significance of this is not clear, especially in view of the apparant minor role MAO might have in snails.

The significance of the small but definite activity of MAO in the snail central nervous system is not clear, especially since other studies[13] have shown re-uptake of the released transmitter to be the probable mechanism of inactivation of DA and 5-HT in the snail. It may well be that MAO has an inactivating function in only a small proportion of nerve cells, but the 'background noise' of the other cells, caused when analyzing whole ganglia, blurs the discovery of this functional significance. However, it could be that MAO has an even distribution, as has been shown for other inactivating enzymes (acetylcholine-esterase[18] and catechol-*O*-methyl-transferase[19]). In this case, the enzyme could be functioning more as an extraneuronal regulating mechanism, guarding against non-specific stimulation by extraneous amines.

MAO activity in various tissues

Tissue	Tissue (nmole/g/min)	Protein (nmole/g/min)
Albumen Gland	ND	ND
Flagella	ND	ND
Radula retractor muscle	ND	ND
Liver	8.053 ± 0.390	62.11 ± 4.05
Kidney	2.506 ± 0.282	25.15 ± 2.81
Salivary gland	3.065 ± 0.268	39.09 ± 3.23
Spermatheca	0.465 ± 0.099	33.41 ± 0.52
Optic tentacle	0.105 ± 0.015	0.80 ± 0.15
Ventricle	0.067 ± 0.001	0.99 ± 0.08
Ganglia		
Buccal	3.253 ± 0.262	26.81 ± 4.30
Supraesophageal	1.502 ± 0.103	16.86 ± 1.07
Suboesophageal	0.904 ± 0.069	13.34 ± 0.81
Suboesophagel[a]	0.189 ± 0.069[b]	2.63 ± 0.96[a]
Suboesophagel[b]	0.415 ± 0.018[b]	5.77 ± 0.25[b]

Tissues analyzed as described in text with tryptamine as substrate except[a] with 5-HT as substrate and[b] with DA as substrate. ND indicates not detected. Values are ± SEM for n between 9-37.

Summary. The distribution and characterization of MAO in various tissues of the snail were analyzed. Only low amounts of the enzyme exist in the different tissues and data suggest that there is more than one type of MAO.

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Phospholipids of *Mycobacterium phlei*[1]

Amongst the phospholipids of mycobacteria, cardiolipin has been reported to be the major component in *M. phlei* (penso)[2], in an unclassified mycobacteria P_6[3] and also in $H_{37}Rv$, $H_{37}Ra$ and *M. avium* (all NCTC) strains[4]. Recently CHANDRAMOULI and VENKITASUBRAMANIAN[5] have reported from this laboratory that the cardiolipin is the major component in *M. smegmatis* and

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