possess at their N terminus sequences which might be split by DPP IV and may, therefore, serve as a natural substrate for this enzyme (e.g., substance P, growth hormone, thyreoliberin).

In their recent communication, Zajac et al. [15] report that the possible role of enkephalin-degrading enzymes and of angiotensin-converting enzyme in meninges could be to maintain the homeostatic concentration of neuropeptides in the CNS and that these enzymes could ensure complete hydrolysis of peptides filtered by the meninges. It seems that DPP IV has a similar functional significance in meninges.

Furthermore, it has been suggested that DPP IV on the brush border of enterocytes and in the proximal tubulus cells of the kidney might constitute the channel and play a significant role in the reabsorption of the dipeptides [16, 17]. It could be deduced that in meningeal cells also, DPP IV may be involved in the digestion of proline-containing peptides, thus making it possible for X-Pro dipeptides to be transported through meningeal cells into the capillaries of the intermediate meningeal lamella.

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Monoamine oxidase activity in single nerve cell bodies from substantia nigra of rat and man

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Summary. In single nerve cell bodies isolated from the substantia nigra of man and rat the active forms of MAO A and MAO B were found by the use of the microdiver technique and specific inhibitors.
Key words. MAO A; MAO B; substantia nigra; rat; man; MPTP.

It has been suggested that MAO B (MAO, monoamine oxidase – monoamine:oxygen oxidoreductase:EC 1.4.3.4.) of the pars compacta of the substantia nigra in humans and primates is responsible for the transformation of MPTP into MPP^+, which is neurotoxic to nerve cells of the pars compacta, producing a Parkinson's disease-like effect [1–5]. Therefore it is possible that rats, which are resistant to MPTP, possess a different pattern of MAO A and MAO B in their substantia nigra as compared to susceptible species, such as man and primates. The microgasometric technique [6] allows the measurement of MAO activity in a single nerve cell body isolated from the pars compacta of the substantia nigra, and by the use of selective inhibitors the activity of both molecular forms of MAO can be determined separately [7].

Methods and materials. Normal albino rats of both sexes, weighing 150–200 g, were used. Rats were decapitated under light ether anesthesia, the brain was dissected and used immediately. Human brain tissue, from patients who died from causes other than neurological ones, was taken at autopsy and kept at 4 °C. The time interval between the death and the MAO assay was 26–30 h. Single nerve cell bodies were identified [8, 9], visualized by lightly staining the cut surface of the brain with 0.5 mM methylene blue, which leaves the glial cells unstained, isolated under a stereomicroscope and dissected free-hand using glass rods with tips of a thin (∅ 15 µm) stainless steel wire [10]. MAO activity was measured microgasometrically at 30 °C using the electromagnetic diver technique [6]. The nerve cell body floating in a droplet of reaction medium was sucked into the diver ampulla, containing a small air bubble, and the microgasometric measurement was performed (for details about the methods [11–16]). The magnetic diver technique allows easier manipulation but yields the same sensitivity and accuracy as the classical microdiver technique (1×10^{-6} µl gas/h) [6, 7]. The enzyme reaction rate was measured by recording oxygen consumption. The reaction medium contained: 1/15 M phosphate buffer pH 7.0, 10 mM tyramine and 10 mM semicarbazide. KCN was omitted from the reaction medium [7]. The total MAO activity was measured in the absence of inhibitors, whereas the activities of the two isoenzymes of MAO were determined in the presence of different concentrations of clorgyline and deprenyl, selective inhibitors of MAO A and MAO B, respectively. In these experiments, an isolated nerve cell body was first preincubated at room temperature for 40 min prior to assay in the medium containing the appropriate inhibitor without the substrate. Readings were taken every 10 min for 1 h. Cell free controls were used to correct for nonenzymic reactions. The changes of gas volume were then converted into pmols of substrate used per h and expressed as the MAO activity per single nerve cell body. The substances used were: tyramine hydrochloride (Hoffmann-La Roche, Basel, Switzerland); semicarbazide hydrochloride (Riedel-De-Haën, Hannover, FRG); clorgyline hydrochloride (May and Baker, Dagenham, UK); l-deprenyl hydrochloride was a gift from Prof. J. Knoll, Department of Pharmacology, Semmelweiss University of Medicine, Budapest, Hungary.

Results and discussion. In single nerve cell bodies isolated from the pars compacta of the substantia nigra in rats and

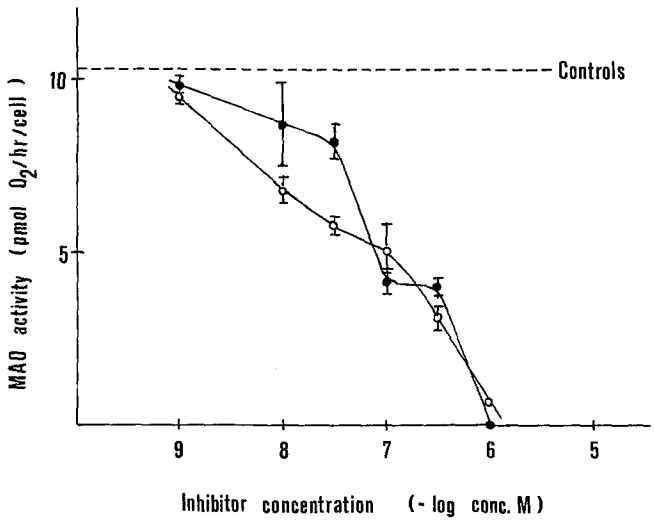

Figure 1. The activity of MAO A and MAO B in single nerve cell bodies, isolated from zona compacta of substantia nigra of the rat and inhibition with clorgyline (○) and deprenyl (●). Each point is the mean of 4–6 determinations ± SD. The hatched line represents the mean activity of MAO in a single nerve cell body without inhibitor (10.26 pmol/h/cell; SD 1.38; N = 10).

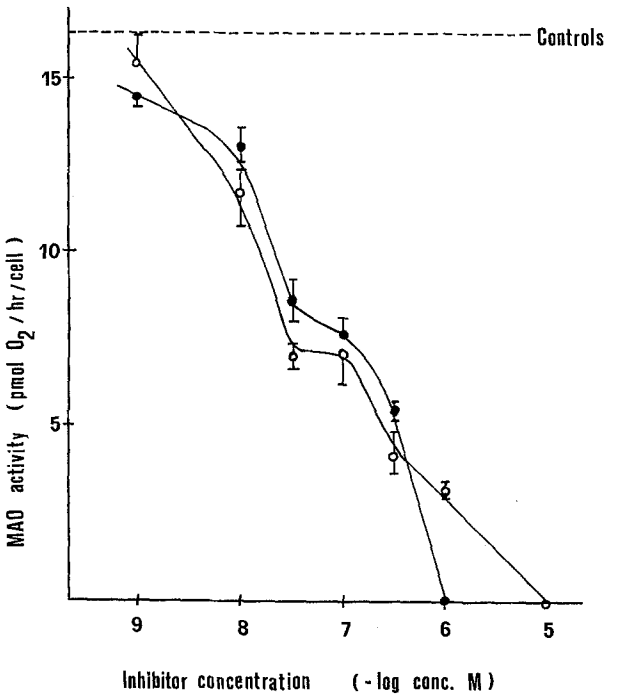

Figure 2. The activity of MAO A and MAO B in single nerve cell bodies, isolated from zona compacta of substantia nigra of man and inhibition with clorgyline (○) and deprenyl (●). Each point is the mean of 4–6 determination ± SD. The hatched line represents the mean activity of MAO in a single nerve cell body without inhibitor (16.3 pmol/h/cell; SD 1.06; N = 10).

humans both types of MAO A and MAO B (figs 1 and 2) were found by using selective inhibitors of individual isoenzymes. Although both inhibitors, clorgyline and deprenyl, show sufficient selectivity for A and B forms of MAO, this selectivity is concentration-dependent, since at high concentrations they lose their specificity [17]. At appropriate concentrations they have been successfully used as model inhibitors for A and B forms, respectively [17]. In our experiments a wide enough range of concentrations of both inhibitors was used to facilitate the distinguishing of individual inhibition curves for MAO A and B. A rather poorly indicated plateau region of the activity vs log inhibitor concentration in figures 1 and 2 suggests that the ratio of both types of MAO could approximate 1:1.

The population of nerve cells from the pars compacta of both the man and the rat appears to be quite uniform as far as their MAO activities are concerned; however, the total activity of MAO in human nerve cells (16.3 pmol/cell/h) was found to be significantly higher than that in the rat (10.3 pmol/cell/h); $p < 0.001$). The difference in post-mortem delay could have been the basis for the observed difference in basal activity of MAO between rat and human nerve cells, although it seems unlikely to us. In two control experiments (4 neurons), rat brain tissue was kept for 30 h at 4 °C but no difference in the MAO activity in comparison to the fresh tissue was found. Moreover, it has been found that MAO of human brain tissue is a very stable enzyme [18, 19].

In single nerve cell bodies isolated from pars compacta of the substantia nigra of the rat, besides MAO A, an about equal activity of MAO B isoenzyme is also present. This observation does not support the idea that the tolerance of the rat towards MPTP is based on the absence of MAO B in the substantia nigra neurons. In agreement with our results on single neurons, a rather high activity of MAO B was found also in tissue homogenates of the substantia nigra of the rat [20]. Recent data locate the MAO B in the serotonergic cells and in astroglia of the rat, but not in catecholaminergic nerve cells [3, 21, 22]. However, it has been reported that MAO B activity is present in noradrenergic nerve cells of the locus ceruleus [7].

For the activities of MAO types in homogenates of human substantia nigra the ratio of about 1:1 [23–25] or somewhat higher in favor of MAO B [26, 27] has been reported. In the substantia nigra of the rat a higher activity of MAO A than that of MAO B was obtained [3, 28]. In our experiments with single nerve cells bodies an approximately 1:1 ratio was found in both human and rat substantia nigra neurons. The discrepancy concerning MAO activities in the rat brain can be explained by the fact that in homogenates glial and other tissue components may contribute to both the total MAO activity and the ratio between the isoenzymes while in single cell experiments only the neuronal MAO activity was measured.

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Effect of acetylhomocysteine thiolactone on nucleolar cytology and lipofuscinogenesis in electric lobe neurons

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Summary. Treatment with acetylhomocysteine thiolactone significantly reduces the cellular level of lipofuscin in neurons of the electric lobe of *Torpedo marmorata*. At the same time, this drug produces a 45% decrease in nucleolar volume in these neurons, reflecting decreased cellular synthetic activity.
Key words. Lipofuscin; nucleolus; nucleolar vacuole; acetylhomocysteine thiolactone; cythiolone.

Lipofuscin, or age pigment, is known to accumulate in the cytoplasm of many cell types in the course of aging [1, 2] and also as a result of environmental stress [3]. Lipofuscin accumulation has generally been regarded as an irreversible build-up of membrane peroxidation products which the post-mitotic cell cannot discard, in the form of inert lysosomal residual bodies [4]. Therefore, the discovery that the drug acetylhomocysteine thiolactone (also known as cythiolone and abbreviated here as CYT) is capable of bringing about a reduction of cellular lipofuscin levels both in vivo [5] and in vitro [6] is of particular interest. Since CYT acts as a free radical scavenger and activates the enzyme superoxide dismutase, this could explain its role in inhibiting lipofuscin formation [7].

The purpose of the present study is to further examine CYT for its effect on the cytology of the cell, particularly the cytology of the nucleolus. Since the size and activity of the nucleolus are well-established markers of ribosome formation and protein synthetic activity of the cell [8, 9] an alteration in nucleolar size produced by CYT could provide new insights into its cellular mode of action. Neurons of the electric lobe of *Torpedo marmorata* were selected for study because of the extensive studies on lipofuscin formation already carried out on these cells [7, 10, 11].

Materials and methods. Animals. Six adult *Torpedo marmorata* collected from the Bay of Naples were used in this study. Their age was estimated to be 1–2 years, based on growth and size parameters established by Aloj Totaro et al. [11]. They were maintained in the laboratory in tanks and fed for 30 days using methods described by Aloj Totaro and Pisanti [10].

Drug treatment. Three animals received daily i.m. injections of acetylhomocysteine thiolactone (cythiolone produced by Roussel-Maestretti, Milano, Italy) at a dosage of 8 mg/kg b.wt for a 30-day period. The remaining three animals received placebo injections for the same 30-day period.

Fixation and preparation of tissue. Prior to sacrifice, the animals were anesthetized by a 15-min immersion in 0.015% MS222 (Sandoz) dissolved in seawater. The aorta was then injected and the animals were perfused with an osmotically balanced buffer as described by Aloj Totaro et al. [7]. Following removal of the blood, perfusion with the fixative, 3.5% glutaraldehyde in a suitable buffer, was initiated [10]. The electric lobes were dissected out and embedded for electron microscopy using standard procedures [10]. Sections were then cut 2-µm-thick on an ultramicrotome, stained with methylene blue and mounted in immersion oil.

Microscopy and measurement of nucleolar diameter. The sections were observed using a Zeiss microscope at ×1600 magnification. An ocular micrometer was placed in the ocular lens, and calibrated using a 1/100 mm Leitz stage micrometer. One ocular micrometer unit was equal to 0.66 µm. The diameter of 30 nucleoli in each animal was measured using the ocular scale. Nucleoli were also scored for the presence or absence of a prominent nucleolar vacuole. Measurements were made only on those cells where the nucleolus appeared to be complete, not in those cells where only a small fragment of the nucleolus was present. Mean nucleolar diameter was first calculated in ocular micrometer units and then converted to µm. Nucleolar volume in μm^3 was calculated using the formula to determine the volume of a sphere, $V = 4/3\,\pi\,r^3$. Determination of the percentage of cytoplasmic area covered by lipofuscin granules had been established by means of electron microscopy in an earlier study [5]. Student's t-test was used to compare nucleolar diameter measurements of the control vs the drug-treated group.

Results. In the table, we present a summary of the cytological parameters measured in neurons of the electric lobe of *Torpedo marmorata*. Measurements from control animals are compared with those from CYT-treated animals. It can be seen that the mean nucleolar diameter of the control animals at 6.81 ± 0.13 µm is significantly larger ($p < 0.001$) than that of the CYT group at 5.58 ± 0.09 µm. When nucleolar volume is calculated, the actual difference in size between the nucleoli of the control and of the CYT-treated animals be-