sponse after exercise in cardiac muscle may indicate that mouse cardiac muscle has an inherent protective mechanism against overstrain due to exertion.

The changes in lipofuscin content did not coincide with the increases in lysosomal enzyme activities. After exercise there was only a slight increase in the lipofuscin content of cardiac and skeletal muscles in the young mice, and none at all in the senescent mice. The accumulation of fluorescent pigments after exercise could be caused by lipid peroxidation associated with heavy exercise. Further studies are necessary to verify this hypothesis.

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Regioselectivity in the oxidative deamination of 2-methyl-1,4-diaminobutane catalyzed by diamine oxidases[1]

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Summary. Diamine oxidase from pea seedlings (PDAO) catalyzes the oxidation of 2-methyl-1,4-diaminobutane in a regioselective fashion, whereas diamine oxidase from pig kidney (KDAO) shows no regioselectivity for the same reaction.

Diamine oxidases (DAO) (E.C. 1.4.3.6., diamine:oxygen oxidoreductase, deaminating) are enzymes which catalyze the oxidative deamination of a variety of diamines[2], including 1,4-diaminobutane (putrescine, Pu) and 1,5-diaminopentane (cadaverine, Ca):

$$H_2N\,CH_2(CH_2)_nCH_2NH_2 + O_2$$
$$\rightarrow H_2N\,CH_2(CH_2)_nCHO + NH_3 + H_2O_2$$
$$n=2: \text{Pu};\ n=3: \text{Ca}$$

Pea seedling DAO (PDAO) and pig-kidney DAO (KDAO) have been among the most extensively studied from plant and animal sources respectively.

Kinetic data on the oxidation of 2-hydroxyputrescine (OH-Pu) and 2-hydroxycadaverine have pointed out certain differences in the active site of these 2 enzymes[3,4]. Indirect findings concerning the regioselectivity came from experiments with OH-Pu, which suggested that both enzymes preferentially attack the amino group more distant from the centre of asymmetry[3].

The aim of this work has been to throw more light on PDAO and KDAO catalytic action, giving new information about their regioselectivity. For this purpose a branched chain diamine, 2-methylputrescine (MePu) was used; MePu was expected to be oxidized by DAO to 2 possible aminoaldehydes, 2- and 3-methyl-4-aminobutanal, both compounds being in equilibrium with the corresponding methyl-Δ^1-pyrrolines. In order to obtain stable derivatives of such reactive compounds, the method of Sakamoto and Samejima[5] seemed the most suitable. According to this procedure, the condensation with 2-aminobenzaldehyde (OAB) and the subsequent oxidation of the formed quinazolinium salt afforded 1'- or 2'-methyl-2,3-trimethylene-4(3H)quinazolone, which were analyzed by ^{1}H-NMR-spectroscopy.

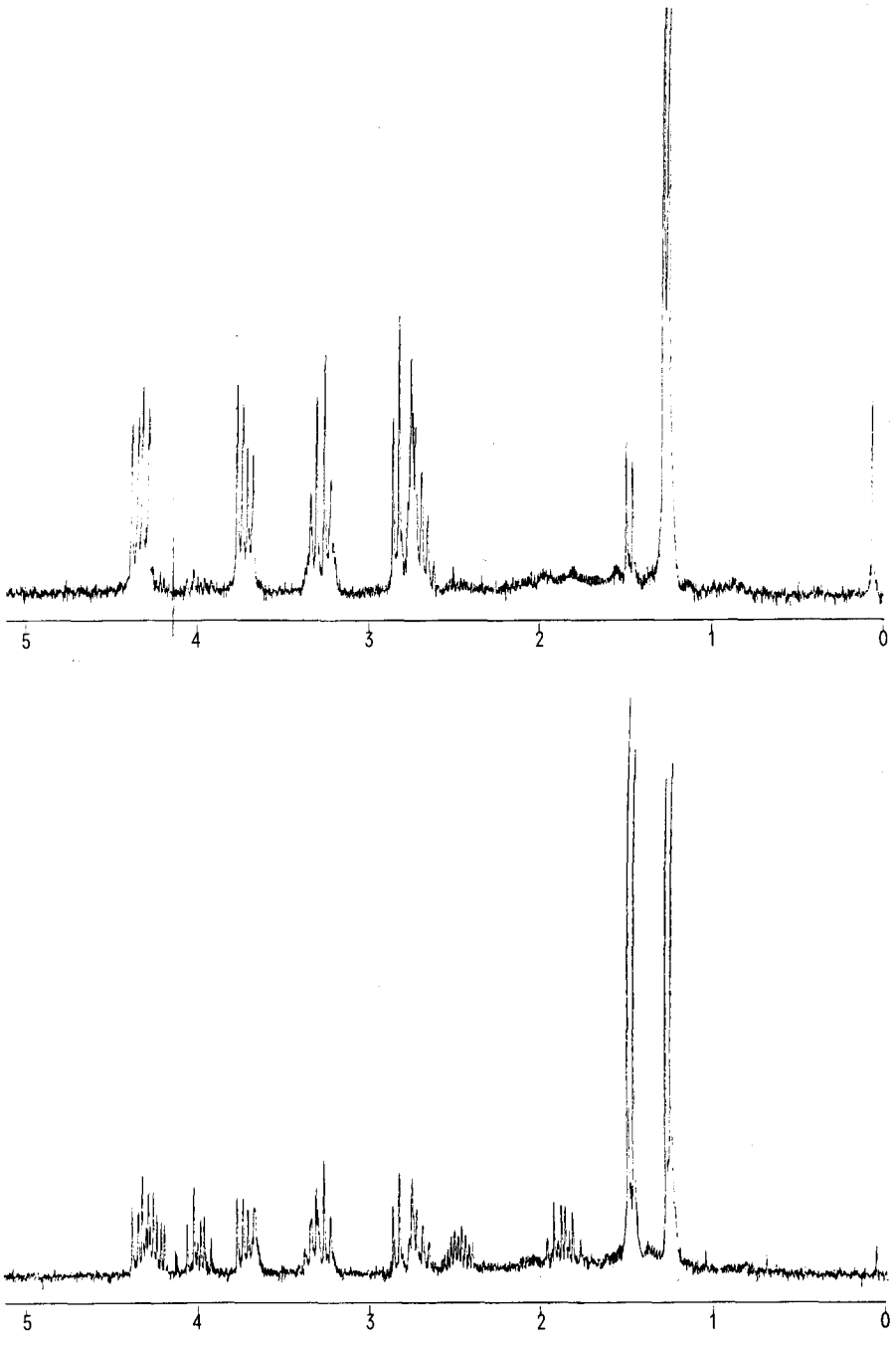
Materials and methods. MePu was prepared from 3-methyladipic acid according to a previously described procedure[6]; OAB was obtained by reducing o-nitrobenzaldehyde with ferrous sulphate[7]. PDAO was extracted from pea seedlings grown in the dark for 10 days, following the method of Hill[8] up to step IV, and then stored at -20 °C in 0.01 M phosphate buffer pH 7.0; KDAO was purchased by Sigma Chem. Co. (USA) and used without further purification.

Enzyme activities were assayed using the colorimetric method introduced by Naik et al.[9], except that the calibration curve was obtained with synthetic Δ^1-pyrroline, produced by acid hydrolysis of γ-aminobutyraldehyde diethylacetal (Aldrich). Methyl-Δ^1-pyrrolines, used for NMR-studies, were obtained by preparative scale incuba-

Specific activity of DAO[a]

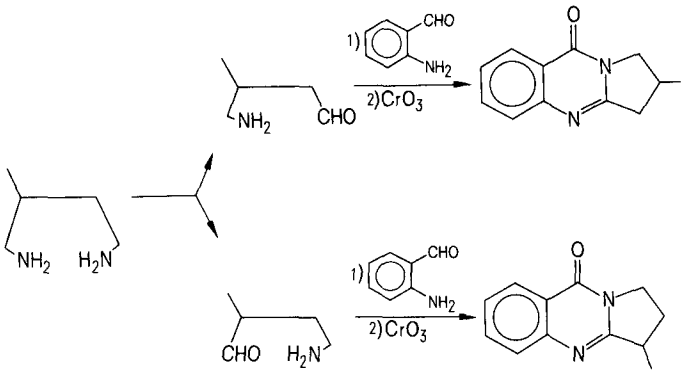
Substrate	Preparation	Enzyme source	
		Pea seedlings	Pig kidney
Pu	1	500	1.1
	2	420	2.2
MePu	1	155 (31%)[b]	0.24 (22%)
	2	117 (27%)	0.60 (27%)

[a] Specific activity is expressed as mU/mg; 1 unit is defined as that amount of enzyme which catalyzes the oxidation of 1 µmole of substrate per min at 37 °C. [b] Percent of specific activity with Pu.



^{1}H-NMR-spectra of aliphatic moieties of the methyl-quinazolones obtained from the products of the oxidations catalyzed by PDAO (upper spectrum) and KDAO (lower spectrum).

tion, at 37 °C for 16 h, 1 U of PDAO or KDAO, 5 mM MePu and 5 mM OAB in 25 mM phosphate buffer pH 7.5 (final volume 50 ml). The reactions were stopped by addition of 10 ml of 10% trichloracetic acid, then OAB was again added to 10 mM. After 2 h at room temperature, the samples were centrifuged and 50 ml of 0.64 M CrO_3 dissolved in 0.8 M H_2SO_4 were added to the supernatants. After 4 h at room temperature, the solutions were made alkaline with 5 N NaOH and extracted twice with an equal volume of benzene. The combined organic phases were concentrated to a small volume at reduced pressure, applied on silica gel plates (0.25 mm thick) and developed with n-butanol-acetic acid-water 4-1-5 (upper phase). The plates were observed under an UV-lamp (254 nm) and the



zone at R_f between 0.6 and 0.7 was scraped off and extracted with chloroform. The purified quinazolones were examined by 200 MHz ^{1}H-NMR-spectroscopy using a Varian XL-200 apparatus.

Results and discussion. In the table are shown the specific activities of the 2 enzyme preparations using Pu and MePu as substrates.

The lower oxidation rates of PDAO and KDAO with OH-Pu suggested that the hydrophilic substitution in the carbon chain between the amino groups is a critical factor[4]. However, from our data it appears that PDAO and KDAO are affected in a similar manner by the substitution of hydrogen on C-2 with a hydrophobic group. Therefore it is very tempting to postulate that steric hindrance rather than polar interactions is primarily involved in determining lower activity with Pu analogs.

In the figure the ^{1}H-NMR-spectra of aliphatic moieties of the methyl-quinazolones obtained from the products of the oxidations, catalyzed by PDAO and KDAO, are reported. Apart from other significant signals, the spectrum of the methyl-quinazolone obtained from the incubation mixture containing KDAO exhibited 2 doublets centered at 1.24 and 1.46 ppm ($J = 8$ Hz) of the same intensity, whereas the product of the incubation of MePu with PDAO had the doublet centered at 1.24 ppm accompanied only by a minor amount ($< 10\%$) of the companion methyl. These data clearly indicate that PDAO is able to oxidize MePu in a regioselective way, whereas the enzyme from an animal source, KDAO, completely lacks regioselectivity. Furthermore the spectrum of the quinazolone from PDAO is consistent with 2′-methyl-2,3-trimethylene-4(3H)-quinazolone[10]. Therefore PDAO catalyzes deamination of MePu to 3-methyl-4-aminobutanal, oxidizing the amino group more distant from the substituted carbon, whereas KDAO is able to oxidize both amino groups without any preference. These results, in addition to the studies with OH-Pu[3], give further information about the regioselectivity of the 2 enzymes. In fact, using the above mentioned hydrophilic analog of Pu, the same behavior appeared for both the enzymes[3]; on the contrary, our results demonstrate that the regioselectivity changes from PDAO to KDAO when a branched chain diamine is used as a substrate.

Hence, it is possible to conclude that in their structural requirements, the active sites of DAO from plant or animal sources are certainly different.

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10 ($CDCl_3$, 200 MHz): 1.24 ppm (2′C−CH_3); 2.70 ppm (2′C−H); 2.80 ppm and 3.30 ppm (1′CH_2); 3.74 ppm and 4.32 ppm (3′CH_2). Also the other tested chemicophysical properties (UV and IR) are in agreement with the structure of the mentioned compound.

Effect of cuprizone feeding on hepatic superoxide dismutase and cytochrome oxidase activities in mice

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Summary. When cuprizone was fed to mice at a 0.5% level for 2 weeks, cupro-zinc superoxide dismutase activity in the liver declined, but there was an increase in mangano-enzyme activity.

Cuprizone (bis-cyclohexanone oxaldihydrazone) is a copper chelating agent used for quantitative determination of the metal[2]. During the course of studies on cuprizone-induced encephalopathy, the formation of giant mitochondria was observed in mouse hepatocytes[3]. In cuprizone feeding studies with weanling mice and rats, many morphological and biochemical assessments of hepatocytes have been carried out to define the causative factor for the genesis of megamitochondria, including estimation of copper-containing enzymes like cytochrome oxidase, amine oxidase and others. However, experiments dealing with another important copper-containing enzyme, superoxide dismutase (SOD), which is present in the eukaryotic cytosol, have not been reported so far in relation to cuprizone feeding to animals. Superoxide dismutases are enzymes that catalyze the conversion of potentially harmful superoxide radicals to H_2O_2 and O_2 and they constitute the primary defence in the cell against O_2 toxicity[4-7]. Superoxide dismutases have been described and in mammals there are 2 distinct classes of these enzymes[4-6]; SOD-1, a cupro-zinc enzyme, sensitive to CN^- and present in the cytoplasm of all cells, and SOD-2, a manganese-containing enzyme, insensitive to CN^- and found primarily in mitochondria. In cupro-zinc SOD, copper is involved in catalysis while zinc maintains the stability of the enzyme[8,9]. The present report pertains to cytochrome oxidase activity of hepatic mitochondria in mice treated with cuprizone for 2 weeks to establish the copper status of the animals. The studies also include results on SOD levels in hepatic cytosol and mitochondria in mice given cuprizone.

Materials and methods. Weanling male mice of the Swiss strain were used throughout the course of this investigation. Cuprizone was mixed with the pulverized laboratory stock diet for experimental mice at a 0.5% level[2] and pair-fed control animals received a similar feed without cuprizone[3]. Both the groups of mice were maintained on the respective diets for 2 weeks, then they were sacrificed by cervical dislocation, and the livers collected and chilled immediately in an ice bath after thorough cleaning. Liver homogenates (10%) were made and mitochondria isolated at $7000 \times g$ in 10 mM HEPES buffer, pH 7.3 containing 220 mM mannitol, 70 mM sucrose, 1 mM EDTA and 0.6% BSA as described[10]. Cytochrome oxidase of mitochondria suspended in 0.25 M sucrose was assessed for its activity.

The assay system contained 80 mM Na-K-phosphate buffer, pH 7.0; 66 mM ascorbate, pH 7.0; 380 μM cyto-