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

A.K. De and M. Subramanian[1]

Biochemistry and Food Technology Division, Bhabha Atomic Research Centre, Bombay-400085 (India), 26 November 1981

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-

chrome c (Horse heart, type VI, Sigma Chemical Co., USA) and 0.1 ml enzyme suspension in a total volume of 1.74 ml. The enzyme activity was determined at 25 °C in a Gilson Oxygraph[11] and represented as nmoles of O_2/mg protein/min.

SOD activity was measured by the pyrogallol method in both mitochondria and 7000 × g post-mitochondrial supernatant, and protein was estimated with the Folin phenol reagent[12,13].

The assay system consisted of 47 mM K-phosphate buffer, pH 7.8 containing 1 mM EDTA; 300 µM pyrogallol in 10 mM HCl and 0.01 ml enzyme preparation in a total volume of 1.0 ml; a cuvette without pyragallol served as blank, The reaction was followed in a Perkin Elmer Double Beam Spectrophotometer Model 124 attached to a recorder at 420 nm[12]. The enzyme activity is expressed in units/g tissue, and 1 unit of the enzyme is defined as that amount required to inhibit the autoxidation of pyrogallol by 50%.

Results and discussion. It was noted that cuprizone feeding caused severe inanition and loss of body weight in experimental mice when compared with ad libitum fed control animals. Rats maintained on pair-feeding give a more reliable indication of the changes in biochemical parameters as compared to that obtainable in animals kept on ad libitum diets and hence pair-fed controls were used in this study[14].

Results on hepatic mitochondrial cytochrome oxidase are given in table 1. It can be observed that the enzyme activity is diminished very significantly by cuprizone feeding (65%) when compared with that of pair-fed controls. Similar results have been reported from other laboratories on this enzyme activity as a result of cuprizone feeding or of copper deficiency[15-17]. However, all the reports are not in agreement about cytochrome oxidase activity in cuprizone-fed mice. Wagner and Rafael[18] did not observe any change in this enzyme activity in mice after cuprizone administration.

The observed discrepancy in the results of different authors could be due to the level of copper remaining unchanged in the liver tissue.

Results on SOD activities of both 7000 × g postmitochondrial supernatant and mitochondria from liver are depicted in table 2. It can be observed from the table that in cuprizone-administered mice, the supernatant enzyme activity decreases by around 26% as compared to that in pair-fed controls. However, in the mitochondrial fraction, there is an enhancement of the activity of this enzyme (40%). A reduction in SOD activity in the rat liver cytosol has been shown in dietary copper deficiency[15]. In other reports, manganese deficiency in mice and chickens was shown to cause a reduction in the level of mitochondrial SOD activity and an increase in the activity of the CN-sensitive enzyme, cupro-zinc SOD[19]. On the basis of these observations, the authors postulate that the 2 forms of the enzyme, although compartmentalized, may not be independently regulated and compensatory changes in their activities could occur, probably on account of the intracellular concentration of superoxide radicals. Other copper-containing enzyme activities like amine oxidase have been shown to decline when cuprizone is added to the diet of animals[20,21]. In cuprizone treatment or in copper deficiency the highest depletion of copper has been shown to occur in the liver, and the mineral level has been shown to decline as a result of cuprizone feeding in several other tissues[22]. It is interesting to note that copper deficiency in the diet does not produce morphological or biological changes similar to those produced by cuprizone feeding, suggesting that simple copper deficiency due to cuprizone administration may not be the only cause for the observed effects of cuprizone in hepatic tissue[23]. It is essential to ascertain whether the observed disturbances in the levels of SOD are related to disturbances in the balance of trace metal ions due to cuprizone administration. Although in the present investigation, assessment of copper concentration in liver was not carried out, the results from our findings and those in the published literature point to a definite deficiency of copper in the livers of cuprizone-treated animals, resulting in decreased activities of copper containing enzymes.

Table 1. Cytochrome oxidase activity in mouse liver mitochondria

Condition	Enzyme activity (nmoles of O_2/mg protein/min)	Activity (%)
Pair-fed control	20.23 ± 1.24 (8)	100
Experimental	7.03 ± 0.43 (8)[a]	35

Values are mean ± SEM. Number of animals in each group is given in parentheses. [a] $p < 0.001$.

Table 2. Superoxide dismutase activity in mouse liver mitochondria and supernatant[a]

Condition	Fraction	Enzyme activity (units/g tissue)	Activity (%)
Pair-fed control	Supernatant	1122.50 ± 57.74 (8)	100
Experimental	Supernatant	833.91 ± 54.10 (8)[b]	74
Pair-fed control	Mitochondria	226.25 ± 16.75 (8)	100
Experimental	Mitochondria	317.73 ± 6.76 (8)[c]	140

Values are mean ± SEM. Number of animals in each group is given in parentheses. [a] 7000 × g post-mitochondrial fraction is termed supernatant. [b] $p < 0.001$; [c] $p < 0.01$.

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