Vitamin A status, glutathione level and microsomal lipid peroxidation in rat lung

	Dietary vitamin A intake		
	Sufficient (n = 20)	Deficient (n = 20)	Excess (n = 17)
Final b.wt (g)	257 ± 8	205 ± 12**	237 ± 6
Total vitamin A in liver (μg/g)	173 ± 20 (7)	[a]4.7 ± 2.9** (7)	823 ± 129** (7)
Serum retinol (μg/dl)	30.8 ± 1.1	16.6 ± 2.2**	47.9 ± 3.9**
Lung wet weight (g)	1.22 ± 0.03	1.21 ± 0.07	1.28 ± 0.07
Protein content[b] (mg/g lung)	80.8 ± 1.8	71.2 ± 3.1*	109.2 ± 5.1**
Glutathione level[b] (μmol/g lung)	1.55 ± 0.08	0.45 ± 0.08**	0.94 ± 0.04**
Microsomal lipid peroxidation[c]	3.00 ± 0.36	13.74 ± 0.90**	1.98 ± 0.30*

Results are quoted as mean ± SE of the mean. Number of animals, shown above each column, applies except where a different number is shown in brackets. Serum retinol results in the subgroups where total vitamin A in liver was assayed were respectively 29.5 ± 1.5, 13.6 ± 1.6 and 46.0 ± 6.9 μg/dl. [a] Includes 4 animals in which total vitamin A in liver was < 0.5 μg/g. [b] Determined in 9000 × g supernatant (postmitochondrial fraction) and expressed in relation to lung wet weight. [c] nmol thiobarbituric acid-reactive products formed per mg microsomal protein per h. * $p < 0.05$, ** $p < 0.01$.

was determined in these tissue digests[3]; and in blood serum of all animals by the method of Hansen and Warwick[8].

All chemicals used in this study were purchased from Sigma Chemical Co., St. Louis, Missouri, USA, or E. Merck, Darmstadt, Federal Republic of Germany. The unpaired Student's t-test (two-tailed) was employed for evaluation of differences between treated and control groups.

Results and discussion. For details of results, see the table. Final b.wt in the vitamin A-deficient group was 20% below control, although the lung wet weights were almost identical. The protein content of the post-mitochondrial fraction of lung homogenate was diminished in the deficient group but increased above control in the vitamin A-loaded group.

Note that glutathione levels were below control in both the experimental groups, whereas lipid peroxidation was enormously increased in the vitamin A-deficient animals and significantly below control in the vitamin A-loaded group.

The results of total vitamin A in liver show that vitamin-deficient and -excess states were in fact achieved.

Dogra et al.[9] have reported that, in vitamin A-deficient rats, the supply of glutathione in lung tissues was inadequate for conjugation of xenobiotics. They proposed that glutathione depletion may be a causative factor leading to chemical carcinogenesis in the lung. However, in extending their experimental work to animals fed with excess vitamin A, which is supposedly capable of suppressing carcinogenesis[10,11], we have found that the glutathione level was diminished rather than elevated or unaltered as might be expected.

When we consider the relationship of lung microsomal lipid peroxidation to vitamin A status a different state of affairs is apparent. Here the vitamin A-deficient and -excess states are quite distinct: there can be no doubt that peroxidation is enhanced in the deficient state and diminished below control in the vitamin A-loaded animals.

We are obliged to conclude that the protective effect of vitamin A against lipid peroxidation in lung microsomes is unrelated to glutathione levels.

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3 Tom, W. M., Fong, L. Y. Y., Woo, D. Y. H., Prasongwatana, V., and Boyde, T. R. C., Chem. biol. Interact. *50* (1984) 361.
4 Montgomery, M. R., and Niewoehner, D. E., J. environ. Sci. Hlth *C13* (1979) 205.
5 Tom, W. M., and Montgomery, M. R., Biochem. Pharmac. *29* (1980) 3239.
6 Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. biol. Chem. *193* (1951) 265.
7 Ellman, G. L., Archs. Biochem. Biophys *82* (1959) 70.
8 Hansen, L. G., and Warwick, W. J., Am. J. clin. Path. *50* (1968) 525.
9 Dogra, S. C., Khanduja, K. L., and Sharma, R. R., Experientia *38* (1982) 903.
10 Saffiotti, U., Montesano, R., Sellakumar, A. R., and Borg, S. A., Cancer *20* (1967) 857.
11 Sporn, M. B., Dunlop, N. M., Newton, D. L., and Smith, J. M., Fedn Proc. *35* (1976) 1332.

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Stereological analysis of lipofuscin in the central nervous system of *Torpedo marmorata:* correlation with superoxide dismutase distribution[1]

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Summary. It was observed that superoxide dismutase activity was inversely proportional to the amount of lipofuscin present in the various anatomotopographical areas of the *Torpedo marmorata* central nervous system. These results support the theory that age pigments are a product of free lipoperoxidation induced by free radicals.

Key words. Lipofuscin; age pigments; *Torpedo marmorata;* free radicals; superoxide dismutase; nervous system.

According to one modern hypothesis[3], lipofuscin is an end product of a lipoperoxidation process triggered by free radicals. It has been shown that in the central nervous system of the batoid selachian *T. marmorata* which is capable of producing electrical discharges, lipofuscin is not distributed equally between the various encephalic regions; the pigment is particularly abundant in the electric lobe[4].

We have recently determined the activity of superoxide dismutase (SOD), an enzyme that neutralizes the toxic action of superoxide radicals, in the central nervous system of *T. marmorata.* In

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Table 1. Stereological parameters studied in CNS of *T. marmorata*. A higher lipofuscin content is observed in the electric lobe

	Forebrain	Optic lobe	Cerebellum	Electric lobe
Average area analyzed per animal ($\mu m^2 \times 10^3$)	2.94	2.94	2.94	2.94
Total average area of lipofuscin granules per animal (μm^2)	12.00	8.53	3.40	48.13
Percent of cytoplasmic area covered by lipofuscin ($\times 10^{-1}$)	4.08	2.90	1.16	16.40

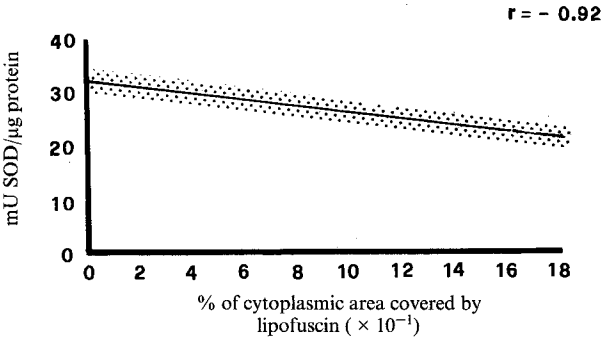
Table 2. Superoxide dismutase activity and percent of cytoplasmic area covered by lipofuscin in the CNS of *T. marmorata*. Note the higher lipofuscin content in the areas with lower SOD activity

	SOD (mU/µg protein)	% of cytoplasmic area covered by lipofuscin ($\times 10^{-1}$)
Cerebellum	34.0	1.16
Optic lobe	28.0	2.90
Forebrain	29.0	4.08
Electric lobe	22.5	16.40

the present work we have assessed more accurately the distribution of lipofuscin in the various areas of the *T. marmorata* brain and in particular we have quantified this by correlation with SOD activity.

Ten specimens of *T. marmorata*, of both sexes and, according to several growth parameters[5] of between 1 and 2 years old, were perfused with Ringer solution until totally bled. The four regions of the CNS were isolated in such a way that half could be processed for electron microscopy using standard techniques and the other half used to determine SOD activity[6]. From the former half, using randomly selected samples, we obtained photographs which were analyzed stereologically to quantify the lipofuscin. For this purpose we utilized an automatic image analyzer system (Ibas I, Zeiss).

The assay of superoxide dismutase was based on its ability to inhibit the autoxidation of epinephrine (0.4 mM) at pH 10.2[7]. In table 1 we show the results of studies of several stereological parameters; the average cytoplasmic area studied per animal, the total average area of lipofuscin per animal and the percentage of the cytoplasmic area occupied by pigment. A higher



Correlation between SOD activity and lipofuscin in the four areas of *T. marmorata*. CNS. y = 31.89–0.59 × ; SD (shaded area) = ±4.5.

lipofuscin content is shown in the electric lobe by the high percentage of the cytoplasmic area covered by the pigment. This parameter is the most interesting in that it gives us on the one hand a relationship between the number and size of the areas of granulation, and on the other hand the cytoplasmic area. Therefore this parameter is used in comparison with the SOD activity of the various regions of the CNS (table 2). To compare these parameters we have calculated the linear regression and the correlation coefficient, which show an inverse proportionality ($r = -0.92$) between high lipofuscin content and low SOD activity (fig.). These results support the theory that lipofuscin is a product of free radicals induced lipoperoxidation[8].

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2 F.A.P. is a 'G. Zuccari', University of Padova, 80/81 Fellow.
3 Donato, H., in: Age Pigments, p. 63. Ed. R. S. Sohal. Elsevier, Amsterdam 1981.
4 Aloj Totaro, E., and Pisanti, F. A., Acta neurol. *34* (1979) 322.
5 Aloj Totaro, E., Pisanti, F. A., Russo, P., and Brunetti, P., Annls Soc. r. zool. Belg., in press (1984).
6 Pisanti, F. A., Frascatore, S., Aloj Totaro, E., and Vuttariello, E., Archs Geront. Geriatr. *2* (1983) 343.
7 Misra, H. P., and Fridovich, I., J. biol. Chem. *247* (1972) 3170.
8 This investigation was conducted at the Zoological Station of Naples. MPI and CNR (Rome) grants are acknowledged.

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Failure of calcium to stimulate Na, K-ATPase in the presence of EDTA[1]

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Summary. The effect of calcium on Na, K-ATPase activity of rat brain homogenates and its modification by the chelating agent EDTA has been investigated. In the absence of EDTA, free calcium (approximately 10^{-6} mol/l) stimulates Na, K-ATPase activity; in the presence of EDTA the same concentration of free calcium is without effect on the enzyme. In the absence of EDTA the stimulation by calcium of Na, K-ATPase activity is enhanced by the additional presence of calmodulin but in the presence of EDTA, even when calmodulin is added to excess, calcium still fails to stimulate the enzyme. The possibility that EDTA interferes with an interaction between a calcium-calmodulin complex and Na, K-ATPase is discussed.

Key words. Na, K-ATPase; calcium; calmodulin.

In a recently published study from this laboratory it was reported that calcium at low concentration stimulates and at higher concentration inhibits neuronal Na, K-ATPase[3,4]. In addition it was shown that stimulation of the enzyme by calcium is modulated by calmodulin. As far as I am aware our report is unique: of those others who have studied the effects of calcium on Na, K-ATPase, all have demonstrated only an inhibition of the enzyme over the effective concentration range of calcium[5–11]. To explain why others have failed to obtain stimulation of Na, K-ATPase by calcium, we[4] suggested that use of the chelat-