through the tumor. Noradrenaline consistently and dramatically lowered the blood flow through various tumors of both the rat[10,11] and the mouse[12], while in the same systems isoproterenol had either a much smaller effect[10,12] or none at all[11]. In contrast, study of the S180 sarcoma (ICRF) showed that interference with energy production caused by L-isoproterenol was much greater than that brought about by noradrenaline, falls in the energy charge[13] being 0.54 and 0.22 respectively[3] after 1 h. If the cutback in energy production resulted largely from decreases in blood flow, then the reverse situation would be expected.

The mechanism of interference of L-isoproterenol with energy metabolism in the sarcoma remains to be elucidated in detail; meanwhile the inhibitory effect of indomethacin pretreatment[9] points to involvement of a dioxygenase and participation of free radicals derived from oxygen[5]. The precise manner in which reactive oxygen species might arise is still a matter for speculation. When acting upon substrate in the presence of NADPH, cytochrome P-450 from rat liver microsomes can produce hydrogen peroxide[14] derived from superoxide[15]. Cytochrome P-450 has also been found in tumors of nonhepatic origin[16,17], but has not apparently been reported in any form of the S180 sarcoma. Moreover, the racemate of isoproterenol, given by the intravenous route in the dog[18,19] and in man[19], was either excreted unchanged or converted enzymically to 3-O-methyl derivatives possessing weak β-adrenergic blocking activity[19]. If the sympathomimetic were to encounter a similar metabolic fate in the mouse, then the chances of tumor injury arising from free radicals produced by cytochrome P-450 acting on the L-isomer would appear to be unlikely. Alternatively, the action of L-isoproterenol might be to increase the proportion of active oxygen species escaping from electron transport particles during oxidative phosphorylation[20].

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The effects of vitamin A nutritional status on glutathione levels and microsomal lipid peroxidation in rat lung[1]

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Summary. In vitamin A-deficient rats, the glutathione level in lung was diminished and microsomal lipid peroxidation much increased. In vitamin A-loaded animals, however, both were depressed below control. Thus vitamin A protection against lipid peroxidation is independent of glutathione.
Key words. Vitamin A; lipid peroxidation; glutathione; lung.

In a recent study[3] we found that peroxidation in rat liver microsomal lipids was inversely related to dietary intake of vitamin A, whereas microsomal cytochrome levels were depressed in both vitamin A-deficient and vitamin A-loaded animals, and certain microsomal metabolic activities were abnormal only in the vitamin A-deficient state. Apparently, the role of vitamin A is multifacetted. Since the lung is known to be especially vulnerable to environmental oxidants[4], we have undertaken another study relating to the effects of dietary vitamin A on the lung.

Materials and methods. The animals were prepared as described previously[3]: in brief, weanling Sprague-Dawley rats weighing initially 50 g were maintained for 90 days on diets providing either 5, 90 or 500 µg/day retinyl acetate. At the end of this period, the vitamin A-deficient group showed signs of moderate hypovitaminosis whilst the vitamin A-loaded group were not noticeably abnormal.

The rats were fasted overnight for 16 h, then lightly anesthetized with ether. Blood was collected from the orbital plexus and the animals were killed by cervical dislocation before removing the lungs. These organs were weighed, washed with ice-cold 0.15 M KCl–0.02 M Tris buffer, pH 7.4, and homogenized in 3 volumes of buffer. The 9000 × g supernatant and microsomal fractions were prepared by differential centrifugation of the resulting homogenate following the procedure of Tom and Montgomery[5]. The protein concentration of the subcellular fractions was measured by the method of Lowry[6]. Glutathione level in the 9000 × g supernatant fraction was determined according to Ellman[7]. Microsomal lipid peroxidation was estimated by the formation of thiobarbituric acid-reactive products in the presence of NADPH and ADP-Fe(III) complex[5].

The liver was also removed from some animals and a portion solubilized in boiling 30% KOH for 15 min. Vitamin A (retinol)

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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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