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# Different Degrees of Lipid Peroxidation in the CNS of Young and Adult Rats Exposed to Short-term Hypobaric Hypoxia

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## Summary

The authors studied the effect of short-term (20 min) hypobaric hypoxia at simulated altitudes of 7000 and 9000 m on the peroxidation of lipids in the cerebral cortex, subcortical formations, medulla oblongata and cerebellum of the laboratory rat. In 5- and 21-day-old rats, increased lipoperoxidation was recorded in all the studied regions of the brain. Differences were observed in sensitivity to the degree of hypoxia. In 5-day-old rats the response to both exposures was the same, but in 21-day-old animals exposure at 7000 m stimulated peroxidation in the cerebral cortex only (at 9000 m in all the parts of the CNS examined). In 35-day-old and adult rats, changes in the malondialdehyde concentration were likewise found after exposure at 9000 m, but not in every compartment (in 35-day-old rats in the cerebral cortex and subcortical formations and in adult rats in the cerebral cortex). In young rats, 30 and 60 min after exposure to hypoxia the malondialdehyde concentration was still higher than in older animals.

### Key words

Hypobaric hypoxia – Lipid peroxidation – CNS development – Rat

## Introduction

In recent years, in association with a study of the pathogenesis of various diseases, considerable attention has been paid to the action of free oxygen radicals in biological systems (Cross 1981). In the organism, 95 % of oxygen is reduced by the cytochrome oxidase system to water, but about 5 % of respiration consists in the univalent transfer of electrons, giving rise to intermediary products which successively appear as the superoxide radical, hydrogen peroxide and the hydroxyl radical (Benedetti *et al.* 1980, Fridowich 1979). These substances are highly reactive and the hydroxyl radical in particular, which has a short half-time ($7x10^{-10}$ s), is extremely reactive (Ahmad *et al.* 1988).

Active forms of oxygen react with nucleic acids, with proteins (they inactivate enzymes) and with lipids, giving rise to physiological or toxic products (Frank and Massaro 1980, Vuillaume 1987).

Unsaturated fatty acids – the basic component of membrane phospholipids – are especially sensitive to the reaction of free radicals (Mead 1976, Tappel 1973, Benedetti *et al.* 1980). The resultant peroxidation of lipids causes shortening of the fatty acid chains and ultimately leads to changes in membrane permeability or to the destruction of cells or of whole cell systems (Frank and Massaro 1980, Braughler 1988).

Since $O_2$ free radicals are produced by the breathing cell under normal conditions, the intracellular peroxidation of lipids is a continuous process, so that membrane structures are in constant danger of being damaged. To maintain the degree of lipid peroxidation at a given, stationary level when the organism is capable of repairing itself, the cells are equipped with a protective enzyme system (superoxide dismutase, catalase, glutathione peroxidase) and natural antioxidants known as "scavengers", which include, for example, alpha-tocopherol, the reduced form of glutathione and ascorbic acid, etc. (McCay *et al.* 1976). In some pathological states, such as ischaemia of the brain, the reactions of oxygen free radicals are intensified (Demopoulos *et al.* 1982, Slater 1984, McCall *et al.* 1987). Tappel (1973) Fridovich (1979) and Tominaga *et al.* (1985) described circumstances in which temporary hypoxia also led to damage owing to the toxic action of oxygen radicals, although it might rather have been supposed that the lowered tissue $pO_2$ would have reduced oxygen toxicity.

As a sequel to our study of the activity of the enzymes of oxidative metabolism following hypoxia (Koudelová and Mourek 1979, Koudelová *et al.* 1979, 1980) and of lipid metabolism, including the proportion of fatty acids in brain tissue and serum (Mourek *et al.* 1986), in the present study we investigated the effect of

short-term hypoxia on the peroxidation of lipids in the tissue of the cerebral cortex, subcortical formations, the cerebellum and the medulla oblongata in rats of several age groups and compared the young and the adult organism.

## Material and Methods

The experimental animals were 155 rats (Wistar strain) of both sexes, of our own breed. Four age groups were studied – 5, 21 and 35 days and adult. The animals were divided into three groups. The first group served as the control. Rats in the second group were exposed for 20 min to acute hypoxia simulated by setting a hypobaric chamber at a barometric pressure of 41 kPa, corresponding to an altitude of 7000 m ($pO_2$ 8.6 kPa). The third group was exposed for 20 min to acute hypoxia at a barometric pressure of 30.7 kPa ($pO_2$ 6.4 kPa), simulating an altitude of 9000 m.

The rats were killed by decapitation. After opening the cranial cavity, the brain was removed and divided on a cooled block into the following parts: the cerebral cortex, subcortical formations (including the thalamus, the hypothalamus and the basal ganglia), the medulla oblongata and the cerebellum.

The peroxidation of lipids was measured immediately after exposing the rats to hypoxia at the relevant altitudes, and in 5-day-old and adult rats at later intervals (30 and 60 min. after exposure at an altitude of 7000 m).

The degree of peroxidation of lipids in the rats brain tissue was determined by the method of Ohkawa *et al.* (1979) for the determination of malondialdehyde (MDA), one of the products formed during the peroxidation of lipids, which is most frequently used for the quantitative evaluation of peroxidation processes. The method is based on the reaction of MDA with thiobarbituric acid (SERVA), in which a coloured product is formed. The best conditions for the reaction are a temperature of 90 °C and pH 3.5. Malondialdehyde-bis-acetal (Merck) was used as the calibration standard. MDA formation was expressed in $ng.mg^{-1}$ tissue wet weight.

The results were evaluted by the Mann-Whitney U test (the Biocybernetics Section of the Department of Physiology).

## Results

In 5-day-old rats, exposure at both the above mentioned altitudes significantly increased the MDA concentration in all the given parts of the CNS compared with the controls. A comparison of MDA production at exposures of 7000 and 9000 m shows a significant difference in the case of cerebral cortex tissue only ($p < 0.05$); changes in the medulla oblongata and subcortical formations were not significant (Fig. 1).

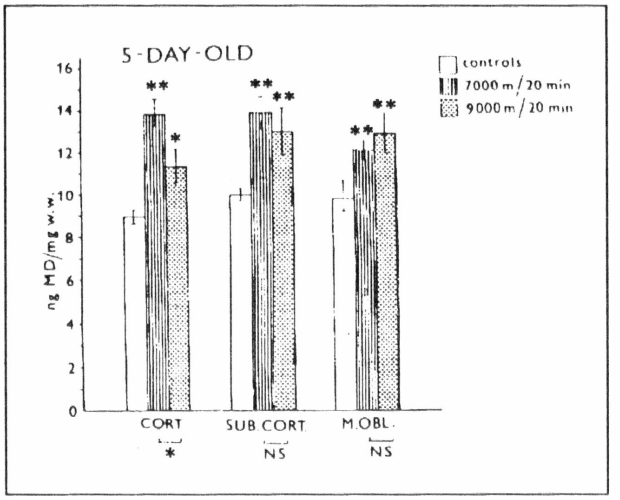


**Fig. 1**

Effect of hypobaric hypoxia on peroxidation of lipids in the cerebral cortex (CORT), subcortical formations (SUB. CORT.) and medulla oblongata (M. OBL.) of 5-day-old rats. y axis: malondialdehyde production in ng/mg tissue wet weight. * difference significant compared with the control at 5 % significance level. ** difference significant compared with the control at 1 % significance level. * significant (or nonsignificant, N.S.) changes at 5 % (*) and 1 % (**) level in comparison with exposure at 7000 and 9000 m. The number of measurements in the individual groups was at least 10.

In 21-day-old rats (Fig. 2), the only significant increase in MDA production after exposure at 7000 m was recorded in the cerebral cortex tissue, but hypoxia corresponding to an altitude of 9000 m produced a statistically significant increase in MDA formation in all the parts of the brain investigated. In comparison of the two exposures, therefore, significant differences in MDA production ($p < 0.01$) were recorded in all the given regions of the brain.

Fig. 3 illustrates the situation in 35-day-old rats. In this group, hypoxia corresponding to an altitude of 7000 m did not produce a significant change in any part of the brain. After 9000 m exposure, however, statistically significant stimulation of peroxidation processes was found in the cerebral cortex and subcortical formations. A comparison of the two exposures shows that the difference was most significant in the cerebral cortex tissue ($p < 0.01$).

In adult rats (Fig. 4), after 7000 m exposure, there were no significant changes in MDA production in any of the given parts of the CNS. Exposure at 9000 m produced significant results in the cerebral cortex tissue only.

Tab. 1 documents the different reaction of the young (5-day-old) and the adult rat organism to short-term hypoxia

**Table 1**

Formation of malondialdehyde in various parts of the brain of 5-day-old and adult rats exposed to an altitude of 7000 m. Malondialdehyde production was measured in ng/mg tissue wet weight.
The results are given as the arithmetical means ± S.E.M.. * difference significant compared with the control for $p < 0.05$, ** difference significant compared with the control for $p < 0.01$. A – immediately after removal of the rats from the hypobaric chamber, B – 30 min after removal from the hypobaric chamber and C – 60 min after removal of the rats from the hypobaric chamber.

| Age | Tissue | n | Control | A | B | C |
|---|---|---|---|---|---|---|
| 5 | Cortex | 15 | 8.98±0.30 | 13.78±0.68** | 13.38±0.54** | 10.45±0.41* |
| | Subcortex | 15 | 9.87±0.34 | 13.78±0.69** | 15.49±0.48** | 11.79±0.38 |
| | Medulla oblongata | 15 | 9.73±0.53 | 12.00±0.45** | 17.02±0.52** | 12.27±0.48* |
| | Cerebellum | 15 | 13.50±1.44 | 15.52±1.18** | 18.49±0.92** | 15.26±0.96* |
| A | Cortex | 10 | 7.97±0.40 | 9.40±0.51 | 10.18±0.30* | 8.78±0.68* |
| | Subcortex | 10 | 8.34±0.42 | 8.32±0.38 | 8.76±0.34 | 8.12±0.69 |
| | Medulla oblongata | 10 | 8.08±0.48 | 8.64±0.57 | 8.68±0.53 | 8.56±0.45 |
| | Cerebellum | 10 | 12.75±0.30 | 13.38±0.52 | 14.28±0.45* | 13.3±0.25 |

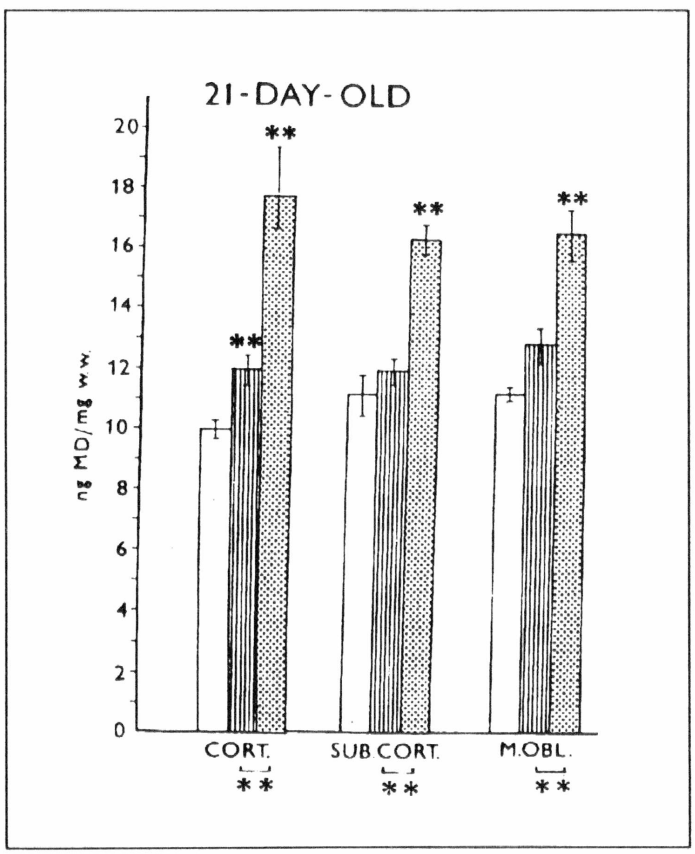


**Fig. 2**
Peroxidation of lipids in various parts of the brain of 21-day-old rats exposed to hypobaric hypoxia at altitudes of 7000 and 9000 m. Further details as in Fig. 1.

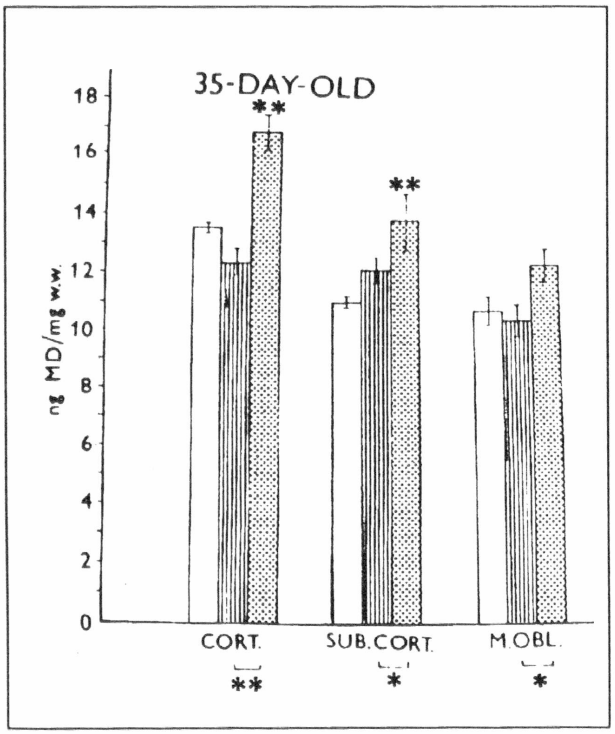


**Fig. 3**
Effect of hypoxia on peroxidation of lipids in various parts of the brain of 35-day-old rats. Further details as in Fig. 1.

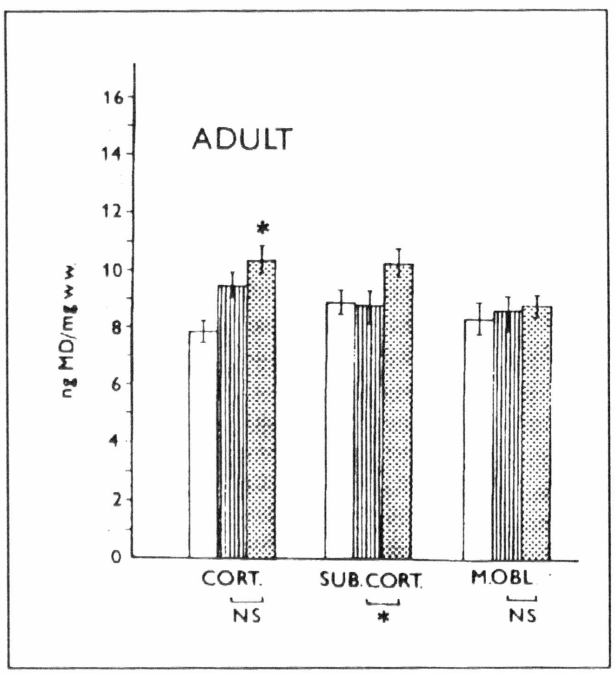


**Fig. 4**
Effect of hypoxia on peroxidation of lipids in the CNS of adult rats. Further details as in Fig. 1.

at a simulated altitude of 7000 m. As demonstrated in Fig. 1, exposure at this altitude was followed by a significant increase in MDA production in all the given parts of the brain, including the cerebellum. Thirty minutes after exposure, the MDA concentration was still significantly higher than in the controls and it was not until 60 min after that the MDA concentration fell again – most significantly in the subcortical formations.

In the adult rat organism, no significant changes were found immediately after 7000 m exposure. After 30 min, statistically significant changes ($p < 0.05$) were recorded in the cerebral cortex and cerebellum compared with the controls, but after 60 min there were again no significant differences in MDA production.

## Discussion

Oxygen deficiency in the CNS can lead to irreversible changes which may eventually cause a loss of some brain functions. During ontogenesis, the organism's resistance to oxygen deficiency changes (Mourek 1958, Trojan 1978). The greatest resistance to oxygen deficiency is displayed by young rats aged about five days and the lowest by 21-day-old animals (i.e. during the weaning period). In addition to these age groups, we chose a group of 35-day-old (i.e. adolescent) rats and a group of adult animals.

It can be seen from Figs. 1-3 that acute (20 min) exposure at 9000 m causes the malondialdehyde concentration to rise in practically all the given regions of the brain of 5-, 21- and 35-day-old rats. In 5-day-old rats, a higher MDA concentration can be seen after a 7000 m exposure, especially in the cerebral cortex and subcortical structures, than at 9000 m. From our point of view, the reason for this disproportion is that practically 100 % of 5-day-old rats survive at 7000 m, whereas survival at 9000 m is only 70 % (the most resistant individuals, in which changes in energy metabolism may not have been triggered as rapidly). MDA production in young rats begins to rise immediately after their exposure to hypoxia and continues to rise 60 min after terminating the exposure (Tab. 1). In adult rats, if MDA production rises at all (in the cerebral cortex and cerebellum), the increase persists for only 30 min after the end of the exposure. The findings in adult rats correlate with the results of Imaizumi *et al.* (1986) who found changes tending towards anaerobiosis in energy metabolism (a raised lactate and pyruvate concentration and a lowered glucose and ATP concentration) in adult male Wistar rats after 3 and 5 min hypoxia. These changes persisted for 30 min after exposure to hypoxia. In all the groups examined, the cerebral cortex (i.e. the phylogenetically youngest region) proved to be the most sensitive to

radical production, while the phylogenetically oldest part – the medulla oblongata – exhibited no changes in MDA concentration, especially in older (35-day-old and adult) rats.

The rate of peroxidation of lipids depends on the degree of non-saturation of phospholipid fatty acids. During maturation, the amount of unsaturated fatty acids in the brain increases (Svennerholm *et al.* 1978, Šmídová *et al.* 1984). *In vitro* experiments in which the non-enzymatic formation of radicals and the subsequent peroxidation of lipids were studied confirmed that the greatest damage to cell membrane structures occurred in neurones with a high unsaturated fatty acid content (Tominaga *et al.* 1985). Our experiments, however, show that the peroxidation of lipids after hypoxia is higher in the immature brain tissue than in the brain of adult animals. This is evidence of differences in the interaction of the protective systems of young and adult individuals which afford protection against lipoperoxidation or act on its development during ontogenesis.

Since lipoperoxidations are initiated primarily by the hydroxyl radical – which is not eliminated in the cells by the protective enzyme system, however (the superoxide dismutase, catalase or glutathione peroxidase) – the role of endogenous and exogenous natural antioxidants such as ascorbic acid and vitamin E is evidently more important. Ascorbic acid is regarded as one of the main endogenous factors protecting the brain cells from the action of radicals (Seregi *et al.* 1978). Changes in the course of ontogenesis (Bien *et al.* 1988, Schreiber *et al.* 1989), in various parts of the brain (Milby *et al.* 1981) and in the ascorbic acid concentration after hypoxia (Schreiber *et al.* 1989) testify that the immature brain tissue is protected not only by a higher ascorbic acid content, but also by faster transport from the plasma to the cerebrospinal fluid (Arad *et al.* 1985, Kratzing *et al.* 1982). Changes in vitamin E levels correlate better with the higher degree of lipoperoxidations in young individuals, but evaluation of the effect of ascorbic acid is hampered by the fact that the rat organism synthesizes ascorbic acid.

Elucidation of the activity of antioxidation systems and their development during ontogenesis could help to reduce the risks both of hypoxia and of subsequent oxygen therapy in premature or perinatally endangered individuals.

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