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No Effect of Hyperbaric Oxygen on Methionine Synthetase Activity in Rats

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Nitrous oxide is the only anesthetic gas or vapor known to inactivate the vitamin B_{12} cofactor of the enzyme methionine synthetase. However, there are conflicting reports on whether oxygen at one atmosphere has any effect. The authors found that the hepatic methionine synthetase activity of Sprague-Dawley rats was unchanged after 4 h exposure to two atmospheres of oxygen. (Key words: Enzymes: methionine synthetase. Oxygen: hyperbaric. Vitamins: B_{12}.)

IT IS WELL ESTABLISHED that nitrous oxide is capable of inactivating the enzyme methionine synthetase.[1–4] This occurs because nitrous oxide is able to oxidize the bound methylcobalamin cofactor of this enzyme from the cob(I)alamin form to the inactive cob(II/III)alamin.[5,6] No other anesthetic agent has been shown to affect methionine synthetase activity (halothane[2,3]; xenon, enflurane, and isoflurane[3]). However, in both these reports, there was some uncertainty over the effect of one atmosphere of oxygen. Deacon *et al.*[2] reported a slight depression of methionine synthesis following a 6-h exposure to 100% oxygen, but only two rats were studied and the activity was not significantly different from controls. Koblin *et al.*[3] on one series of mice found a 44% reduction in activity after 4 h exposure to 0.8 atmospheres of nitrogen plus one atmosphere of oxygen. This achieved statistical significance, but these results were not repeatable.

In our view, further studies at one atmosphere of oxygen could not resolve the uncertainty created by the conflicting studies that already have been published. Therefore, we have exposed rats to two atmospheres of oxygen for 4 h. If oxygen does inactivate methionine synthetase by oxidizing the vitamin B_{12} cofactor, in a similar manner to nitrous oxide, then the greater the partial pressure of the gas, the greater should be the tendency to oxidation. We exposed the rats to two atmospheres because it is the highest partial pressure at which there is no danger of oxygen toxicity.

Methods

Adult male Sprague-Dawley rats were supplied specified-pathogen-free from the Division of Comparative Medicine at the Clinical Research Centre. Rats were exposed in groups of three in a 20-l steel pressure chamber.[7] For the pressure group, the chamber was flushed thoroughly with oxygen at one atmosphere pressure, after which oxygen was added to give a total pressure of two atmospheres absolute. After 4 h exposure to hyperbaric oxygen, the chamber was decompressed over 5 min. For the control group, the chamber contained air at atmospheric pressure. The temperature in the chamber was kept at 23.5 ± 2°C, carbon dioxide was removed by sodalime, and excess water vapor was removed by silica gel.

After exposure, the animals were anesthetized with pentobarbitone, exsanguinated, and their livers removed and stored at −20°C. The hepatic methionine synthetase activity was determined by the method described by Deacon *et al.*[2] and expressed as nanomoles of methionine formed per hour per milligram of protein.

Results

The mean value of hepatic methionine synthetase activity in the rats exposed to two atmospheres of oxygen for 4 h was 91% of the value in the controls (table 1). The difference was not significant by Student's two-tailed t test ($P > 0.2$).

Discussion

Because nitrous oxide acts as an oxidizing agent in its reaction with vitamin B_{12}, it is at least theoretically possible that oxygen at high partial pressures might have a similar action. Furthermore, the *in vitro* estimation of methionine synthetase activity requires a reduced environment and it is possible that high oxygen concentration might affect the *in vivo* activity of the enzyme. This might explain the conflicting reports of the effect of one atmosphere of oxygen on methionine synthetase activity.

TABLE 1. Hepatic Methionine Synthase Activity

	Two Atmospheres of Oxygen	One Atmosphere of Air
No. of rats	9	6
Mean methionine synthetase activity ($nmol \cdot h^{-1} \cdot mg\ prot^{-1}$)	3.11	3.41
SD	0.52	0.48
SEM	0.18	0.19

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We selected 4 h exposure to oxygen at an absolute pressure of two atmospheres as the upper limit that was free from danger of oxygen toxicity and it seems very unlikely that more prolonged exposure would produce a different picture. The findings in this experiment are unequivocally negative, and we conclude that nitrous oxide remains the only therapeutic gas or vapor that is known to inhibit methionine synthetase activity.

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