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FORMATION OF FREE RADICAL INTERMEDIATES DURING NITROUS OXIDE METABOLISM BY HUMAN INTESTINAL CONTENTS

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We have demonstrated that nitrous oxide (N_2O) is metabolized by bacteria present in the intestine of both humans and rats (1). A recent epidemiological study of dental professionals suggests an association between long-term occupational exposure to N_2O and an increased incidence of congenital abnormalities and spontaneous abortion (2). N_2O has been shown to be a potent teratogen in rats (3,4). A possible link between these studies is that metabolism of N_2O may produce teratogenic or carcinogenic by-products. The end-product of reductive metabolism of N_2O by intestinal bacteria, however, is inert nitrogen gas. Therefore, if metabolism is associated with toxicity, it would most likely be due to the production of transient reactive intermediates or their secondary reaction products that subsequently enter the blood. Free radicals have been shown to be intermediates in the metabolic reduction of other nitrogen compounds (5) capable of inducing both teratogenicity and carcinogenicity. Irradiation of an aqueous solution of N_2O by X-rays followed by addition of this solution to Escherichia coli resulted in the killing of the bacteria. This killing was ascribed to production of long-lived free radicals derived from N_2O radicals (6). We report here preliminary evidence for the existence of such free radicals during metabolism of N_2O by human intestinal contents.

To accumulate sufficient concentration of these free radicals for detection by electron paramagnetic resonance (E.P.R.), incubations of N_2O with human intestinal contents were performed in the presence of a spin trap (7). Human intestinal contents (200 mg) were dispersed in 2 ml of 0.1 M potassium phosphate buffer, pH 7.5. This mixture was degassed with argon, saturated with N_2O, and incubated at 37° in the presence of a 10 mM concentration of the spin trap 5,5-dimethyl-1-pyrroline-N-oxide (DMPO). Control suspensions were incubated under argon. The trapped radicals were extracted and concentrated. Complex E.P.R.-spectra suggesting several trapped radicals were obtained after a 5 hr incubation in which anaerobiosis was produced by β-D-glucose, glucose oxidase, and catalase. Spectra obtained from incubations under N_2O were of greater integrated intensity. However, when a less strictly anaerobic incubation was performed for 20 hr under an argon atmosphere, but without β-D-glucose, glucose oxidase, and catalase, a spectrum characteristic of hydroxyl radical adducts was obtained (Fig. 1). The incubation with N_2O produced from three to twenty times higher concentrations of trapped free radicals than the argon control in paired experiments using four different specimens. A similar result was obtained with an alternate spin trap, phenyl-N-tert-butylnitrone (PBN).

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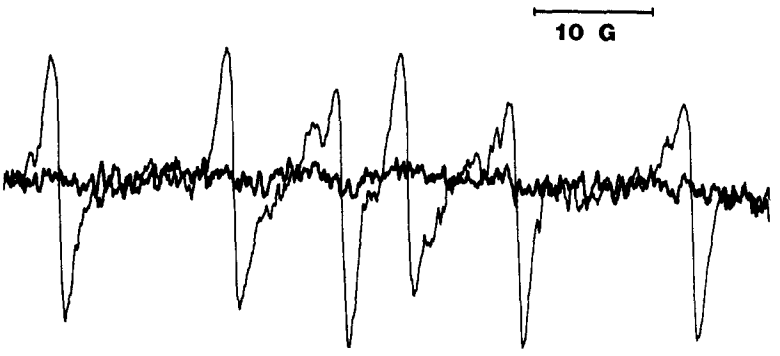

Figure 1. E.P.R. spectrum of free radicals trapped by DMPO in human intestinal content incubations. The spin trap adduct was extracted in ethyl acetate and the spectrum measured under argon at 25° C with a Varian E-104A spectrometer. Amplification was the same for both spectra; the higher amplitude trace is from the N_2O incubation, the lower amplitude trace is from the argon control.

To differentiate between production of free radicals from N_2O and inhibition of destruction of trapped radicals by N_2O, we prepared the hydroxyl radical-PBN adduct chemically and determined its stability under N_2O or argon atmospheres in the described incubation mixture. N_2O was shown to have no effect on reduction or destruction of hydroxyl radical-PBN adducts once they were formed.

These results suggest that the spectra of the hydroxyl radical adduct observed after 20 hr are the product of hydrolysis of less stable N_2O radical adducts rather than due to an increased endogenous production of hydroxyl radicals stimulated by the presence of N_2O. The formation of N_2O radicals in the body may be a basis for some of the observed toxic effects of this agent.

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