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Chain Elongation and Desaturation of Palmitic Acid in Liver Microsomes of Rats Subjected to Hyperbaric Exposure[1] (39225)

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An increase in the amount of long-chain fatty acids in liver glycolipid of rats subjected to a hyperbaric exposure and fast decompression has been reported (1). A similar observation was reported in the glycolipids of liver, kidney, and spleen from rats exposed to 20 atmosphere-absolute (ATA) of $He-O_2$ when animals were compared to those held at 1 ATA of $He-O_2$ (2). An attempt was made to correlate the alteration in the amount of fatty acids and the enzyme activity involved in fatty acid chain elongation and desaturation under hyperbaric exposure. This paper is a report on the enzyme activities associated with chain elongation and desaturation of palmitic acid in liver microsomes of rats exposed to 20 ATA of $He-O_2$.

Materials and methods. Simulated diving experiments were performed according to the procedure described by Bitter and Nielsen (3). Male Sprague-Dawley rats, with an average weight of 200 g, fed regular lab chow were subjected to a hyperbaric exposure of 20 ATA ($He-O_2$) for 12 hr and subsequently to a 7-hr decompression schedule. Two control groups of animals each were held in ambient air and 1 ATA of $He-O_2$.

Following decompression, the animals were anesthetized with ether and bled. The livers were removed immediately, homogenized with a Sorvall Omni-mixer at half speed for 5 sec in 2 vol of 0.25 *M* sucrose–5 m*M* $MgCl_2$ solution, and then strained through a fiber screen. The cells were then further homogenized with a Dounce homogenizer (six strokes). The homogenate was centrifuged at 17,000*g* for 30 min. The microsomes were separated by centrifuging the supernatant fluid at 105,000*g* for 2 hr. The microsomal pellet was suspended in the homogenizing medium. The protein content of the microsomes was measured by the method of Lowry *et al.* (4).

The incubation conditions for the enzyme assay were described by Sprecher (5). The incubation mixture contained 1.6×10^5 cpm of palmityl-1-[^{14}C]CoA (58 μCi/mg), 5 mg of microsomal protein, 10 μmole ATP, 2 μmole NADPH, 0.2 μmole CoA, 0.2 μmole Malonyl-CoA, and 10 μmole $MgCl_2$ in 1.5 ml of 0.1 *M* potassium phosphate buffer, and was incubated at pH 7.4 for 30 min at 37°. Following the incubation, the lipids were extracted and converted to methyl esters with 5% HCl–MeOH. The methyl esters were separated and collected from a Beckman 2A gas chromatograph equipped with a thermal conductivity detector employing a 240 × 0.32 cm aluminum column packed with 15% ethylene glycol succinate–silicone complex on 100/120 mesh Gas Chrom P. The fatty acids were collected and dissolved in a toluene-based scintillation fluid containing 0.55% Permablend 1 (Packard Instrument Co., Inc.). The radioactivity was counted in a Packard liquid-scintillation spectrophotometer, and the percentage of conversion of the substrate was calculated. The significance of the comparative data was calculated using a Student's *t* test.

Results and discussion. It is well established that long-chain fatty acids are chain elongated (6) and desaturated (7) in the microsomal fraction of rat liver. When pal-

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TABLE I. THE EFFECT OF A HYPERBARIC ATMOSPHERE ON THE CHAIN ELONGATION AND DESATURATION OF PALMITIC ACID USING LIVER MICROSOMES FROM RATS EXPOSED TO 20 ATA OF HE-O_2.[a]

Fatty acid	1 ATA of air (n = 4)	1 ATA of He-O_2 (n = 3)	20 ATA of HE-O_2 (n = 9)
16:0[b]	77.6[c] ± 2.3	86.4 ± 4.1	82.0 ± 1.6
16:1	8.1 ± 1.3	6.2 ± 1.7	3.4 ± 0.5[d]
18:0	11.0 ± 1.4	5.4 ± 1.6[e]	12.7 ± 1.0[f]
18:1	3.2 ± 0.9	1.9 ± 0.9	1.9 ± 0.3[g]

[a] The distribution of radioactivity in the fatty acids, following the incubation of 1.6×10^5 cpm palmityl-1-[^{14}C]CoA (58 μCi/mg) with 5 mg microsomal protein, 10 μmole ATP, 2 μmole NADPH, 0.2 μmole CoA, 10 μmole $MgCl_2$ in 1.5 ml of 0.1 *M* potassium phosphate buffer, pH 7.4, at 37° for 30 min. n = Number of animals measured.

[b] Number of carbon atoms in fatty acids:number of double bonds.

[c] Relative percentage of radioactivity; values are the mean ± standard error.

[d] Significantly different from 1 ATA of air and 1 ATA of He-O_2 controls ($P < 0.001$; $P < 0.025$).

[e] Significantly different from 1 ATA of air control ($P < 0.025$).

[f] Significantly different from 1 ATA of He-O_2 control ($P < 0.001$).

[g] Significantly different from 1 ATA of air control ($P < 0.05$).

mityl-1-[^{14}C]CoA was used as substrate and incubated with the liver microsomes from control animals maintained in air, 77.6% of the radioactivity was in palmitate, 8.1% in palmitoleate, 11.0% in stearate, and 3.2% in oleate (Table I). These data agree with the results of Sprecher (5), except that the incorporation of activity of ^{14}C into stearate was lower in our experiments. This difference may have resulted from use of a fat-free diet in the animals used by Sprecher.

Chain elongation of palmitate to stearate for rats held at 1 ATA of He-O_2 was depressed significantly ($P < 0.025$) as compared to the animals held at 1 ATA of air (Table I). The desaturase activity was also slightly decreased in helium control animals but the differences were not significant. These findings support our previous observation that helium can affect cellular metabolism at ambient pressure (2). When rats were exposed to an environment of 20 ATA of He-O_2, the percentage of chain elongation from palmitate was about the same as for rats held at 1 ATA of air and was two times greater than for the animals held at 1 ATA of He-O_2 ($P < 0.001$, Table I). Sprecher, and Goldberg *et al.*, have shown that hepatic microsomes contain more than one fatty acid chain elongation system (5, 8); therefore it is important to choose the appropriate substrate for use in the detection system to determine the changes in enzyme activity involved in chain elongation of fatty acids. The desaturase activity for animals held at 20 ATA of He-O_2 was suppressed as compared to the air and helium control animals ($P < 0.001$; $P < 0.025$ for 16:1; and $P < 0.05$ for 18:1). It has been reported that the microsomal desaturation activity is affected by various physiological conditions of the animals such as age (9), hormonal status (10), and dietary conditions (11, 12). Also, microsomal chain elongation of fatty acid is affected by genetic disorders (8) and dietary conditions (5, 13). Our data suggest that hyperbaric exposure can affect the microsomal chain elongation and desaturation of fatty acids in rat liver, but the meaning of the functional change at this time is unknown.

Summary. The enzyme activities associated with chain elongation and desaturation of fatty acid in hepatic microsomes from rats held at 1 ATA of air, 1 ATA of He-O_2, and 20 ATA of He-O_2 were studied. It was found that both the microsomal chain elongation and desaturation of fatty acids were depressed in rats held at 1 ATA of He-O_2 as compared to animals held at 1 ATA of air. When animals were exposed to an environment of 20 ATA of He-O_2, the chain elongation of fatty acid was about the same as for rats held at 1 ATA of air and was two times greater than for the rats held at 1 ATA of He-O_2. The desaturase activity was depressed as compared to the two groups of control animals held at 1 ATA of air and 1 ATA of He-O_2.

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