Decompression sickness risk reduced by native intestinal flora in pigs after H_2 dives

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Kayar SR, Fahlman A. Decompression sickness risk reduced by native intestinal flora in pigs after H_2 dives. Undersea Hyper Med 2001; 28(2):89–97.—Decompression sickness (DCS) risk following a simulated dive in H_2 was lower in pigs with a native intestinal flora that metabolized H_2. Pigs (n = 27; 19.4 ± 0.2 kg body mass) were placed in a chamber that was pressurized to 22.2–25.5 atm (absolute; 2.2–2.6 MPa) with 84–93% H_2 for 3 h. Chamber concentrations of O_2, H_2, He, N_2, and CH_4 were monitored by gas chromatography. Release of CH_4 from the pigs indicated that intestinal microbes had metabolized H_2. After decompressing to 11 atm, the pigs were observed for DCS. Animals with DCS released significantly less ($P < 0.05$) methane (0.53 ± 0.37 ppm CH_4; n = 5) than those without DCS (1.40 ± 0.17 ppm CH_4; n = 22). The DCS risk reduction was attributed to the loss of roughly 12% of the total volume of H_2 that could be stored in the tissues of the pigs. Thus, H_2 metabolism by the native intestinal flora of pigs may protect against DCS following a simulated H_2 dive.

biochemical decompression, decompression illness, hydrogen diving, Methanobrevibacter smithii, *methanogens*

We report here the serendipitous finding that the native intestinal flora of some pigs may confer a measure of protection against decompression sickness (DCS) following exposure of the pigs to hyperbaric H_2. This protective effect is attributed to a process for which the name "biochemical decompression" has been coined (1).

Deep diving to depths exceeding 300 meters seawater (msw; 3 MPa) with H_2 as a major component of the breathing mixture offers some practical advantages over helium or nitrogen. The most notable advantages are ease of ventilation at high pressures and narcotic suppression of high pressure neurologic syndrome (2–4). However, conventional decompression from deep dives with any of these gases requires more than a week to reduce DCS risk to acceptable levels. Biochemical decompression offers the possibility of significantly shortening conventional decompression time. We have shown (1) that rats given intestinal injections of the H_2-metabolizing microbes *Methanobrevibacter smithii* had a lower incidence of DCS than untreated rats. The H_2 metabolism of these microbes is described as:

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \qquad \text{Eq. 1 (5)}$$

By measuring methane release rate from the rats, we computed that the treated rats lost a volume of H_2 equivalent to an estimated 5% of the total volume of H_2 stored in their tissues during a hyperbaric H_2 exposure. The elimination of this small but critical volume of H_2 reduced the incidence of DCS in these rats by more than 50%, using a chosen compression and decompression sequence (1). However, gas flux kinetics in a small animal are expected to be considerably faster than those in a larger animal, consistent with generally faster kinetics of physiologic processes in small vs. large animals (6). The faster uptake and loss of gases in smaller animals raises the possibility that biochemical decompression is practicable only in small animals. Consequently, in preparing to advance biochemical decompression for use by human divers, this concept was tested in 20-kg pigs to study an animal model closer in size and cardiovascular physiology to humans (7).

Pressurization and depressurization of a dry hyperbaric chamber with H_2 was used to simulate a H_2 dive. Our experimental design required that we identify a baseline sequence of pressures that would cause a high incidence of DCS in untreated control animals. This would afford the opportunity for a treatment with H_2-metabolizing microbes to significantly reduce DCS risk. We selected compression and decompression rates of 0.45 atm · min^{-1} (0.045 MPa · min^{-1}), and duration at maximal pressure of 3 h arbitrarily. Maximal pressure for this baseline dive was to be determined by a simple and efficient trial process (E. C. Parker, personal communication 1996; and 8) in which a small number of animals (in our case, 5) would be tested at one arbitrarily selected pressure. The selection of a higher or lower pressure for subsequent dives with other small groups of animals would be chosen on the basis of DCS incidence from the preceding

group. If very few animals in the group (0, 1, or 2 in our case) had DCS, then the next group would be tested at a deeper depth, and the converse if all animals had DCS. Pressures would be tested iteratively until a pressure was found that generated a 70–90% DCS incidence following decompression in these untreated animals. Some idea of the pressure range to investigate was provided by a study with rats breathing 98% H_2, in which DCS incidence varied from 0% at 18 atm to 100% at 28 atm (9).

We followed this procedure with five animals at three different pressures within our range, and found that something unanticipated was occurring: the DCS incidence did not increase with increasing pressure. However, a pattern appeared to be emerging that was consistent with our concept of biochemical decompression. By including more animals in the study and statistically analyzing the data, we determined that animals that released readily detectable quantities of methane had a lower risk of DCS than those animals that released little or no quantifiable methane. Methane could only be released from these animals as a product of H_2 metabolism by their native intestinal flora. Thus, this study supports the idea that the H_2-metabolizing capacity of the native intestinal flora of some pigs is sufficient to elicit a reduction in DCS risk from a hyperbaric H_2 exposure. This outcome was unanticipated because we expected that H_2 biochemical decompression would be demonstrable only by supplementing the intestinal flora with high concentrations of methanogens. The findings of this study have implications for the health of people currently engaged in H_2 diving, as well as for future applications of biochemical decompression in human diving.

MATERIALS AND METHODS

Animals and training: Pigs (*Sus scrofa*, neutered or intact juvenile male Yorkshires, $n = 27$, mean body mass ± 1 SEM = 19.4 ± 0.2 kg) were used for all experiments. The pigs were housed before experiments in an accredited animal care facility and had ad libitum access to water. The pigs were fed once daily with laboratory animal chow (Harlan Teklad, Madison, WI; 2% by body weight). All procedures were approved by the Institutional Animal Care and Use Committee.

Animals were trained to walk at a moderate pace (50–60 m · min^{-1}) for intervals of 5 min on a treadmill in the laboratory, separated by intervals of 5 min rest, to acclimate them to a treadmill and to verify that this workload was not excessively strenuous. They were then trained to walk at a slower pace (30 m · min^{-1}) for intervals of 5 min inside the compression chamber in 1 atm air, on a treadmill that constituted the floor of the chamber. The pigs were acclimated to being left confined and unattended in the chamber, where they were free to lie down. They were trained to rise and walk whenever the treadmill was activated. Treadmill performance was subsequently used to help evaluate the animals for DCS.

Dive protocol: The hyperbaric chamber (5.7 m^3 internal volume, WSF Industries, Buffalo, NY) and the facility in which it stood were specially designed for safe handling of high pressures of H_2 (1). View ports on the chamber, as well as a video camera aimed through a view port, allowed ad libitum viewing of the animal from multiple angles throughout the experiment.

For each experiment, one pig was placed in the compression chamber, standing on the treadmill. Transparent plastic panels kept the animal confined to an area of 45 cm × 100 cm and a ceiling of 45 cm, but with sufficient open areas within the panels to allow free ventilation of the pig's space. At the front of this enclosure were troughs for food and water. The back wall of this enclosure was fitted with tubes that were connected to a port on the chamber. The port was plumbed to send a stream of gas from the chamber through a flow meter and then to a gas chromatograph (GC; model 5890A series II, Hewlett-Packard Co., Wilmington, DE). Analysis occurred automatically every 12 min. The gases analyzed were He, H_2, O_2, N_2, and CH_4. The commercially purchased supplies of He, O_2, and H_2 were certified and confirmed to be below 0.1 ppm CH_4. A gas blower kept the gases in the chamber well mixed, as confirmed by replicate gas chromatographic analyses of samples drawn from the pig's enclosure and from a second, relatively distant location within the chamber.

The flow meter for analyzing chamber ventilation rate was calibrated with pure He, H_2, O_2, and N_2, and with air, 2% O_2 in He, and 2% O_2 in H_2, using a water spirometer. Ventilation rate estimates were reproducible within 2–4%. Linear interpolation based on relative percentages of gases was used to estimate the ventilation rates of all other gas mixtures. The mean ventilation rates used in these experiments at final chamber gas composition were either 115 liter (STP) · min^{-1} or 410 liter · min^{-1}. For ease of subsequent data analysis, all methane data collected at a ventilation rate of 410 liter · min^{-1} were normalized to a flow rate of 115 liter · min^{-1}. The flow meter was checked frequently during the experiment to assure that the ventilation rate was stable and independent of chamber pressure. Chamber ventilation rate was further confirmed by analysis of helium washout from the chamber. The volume of the sampling line from the chamber to the GC was estimated to be less than 1 liter. Thus at the flow rates used, the sample line was completely flushed more than 100 times per minute.

The chamber was pressurized with He to an absolute

pressure of 11 atm, adding O_2 as necessary to replace O_2 consumed by the pig or lost by the ventilation of the chamber. Initial pressurization rate was selected by appearance of comfort for the animal's ears (ca. 0.15 atm · min^{-1} for the first 2–3 atm, up to 0.45 atm · min^{-1} at greater pressures). This initial pressurization with He caused a dilution of O_2 in the chamber from approximately 21% at 1 atm O_2 (0.2 atm P_{O_2}) to 2% O_2 at 11 atm (0.2 atm P_{O_2}). The dilution of O_2 was necessary for the subsequent safe addition of H_2 to the chamber gases (1) because mixtures of H_2 and O_2 are combustible if the O_2 concentration exceeds 5% (10). The chamber was then flushed with H_2 while maintaining the chamber at a constant pressure of 11 atm, to a concentration of 60–75% H_2, adding O_2 as necessary to maintain normoxia (0.2 atm P_{O_2}). This process required approximately 30–40 min.

The chamber was then further pressurized with H_2 and O_2 to final pressures of either 22.2 atm, 24 atm, or 25.5 atm total pressure, at a rate of 0.45 atm · min^{-1}. Animals remained at the maximal pressure chosen for the experiment for 3 h, with less than 1% drift in selected pressure during that time. The O_2 partial pressure was maintained essentially constant throughout this portion of the dive, at the slightly elevated levels (0.3–0.5 atm P_{O_2}) that are customary for respiration in hyperbaria (4). However, the concentration of H_2 slowly increased over the period of hours spent at maximal pressure by a few percentage points as chamber gases containing traces of He and N_2 were replaced with H_2 during chamber ventilation. The percent of H_2 and partial pressure of H_2 reported (Table 1, Figs. 1 and 2) were from the final 36 min at maximal pressure (mean and standard error of three chromatographic reports).

After 2.5 h, the chamber treadmill was remotely activated. The animals were made to walk for 5 min to observe their gait and to judge that they appeared normal while compressed.

Three hours (± a few seconds) after arriving at maximal pressure, decompression occurred at a rate of 0.45 atm · min^{-1} to 11 atm, while observing the animals closely. When chamber pressure reached 11 atm, animals were observed as they walked (30 m · min^{-1}) on the chamber treadmill for intervals of 5 min, followed by a 5-min rest. The treadmill observation continued for up to 1 h or until signs of severe DCS were noted and agreed upon by at least three observers. These signs were primarily neurological, including limb dysfunction, falling, difficulty standing or righting after falling, and seizures. Occasionally animals with clear neurological symptoms also had labored breathing, which we interpreted as a possible sign of cardiopulmonary DCS in addition to neurological DCS. Some animals were observed to have signs of skin DCS (conspicuous lavender to dark purple mottling of the skin, with or without itching), but skin DCS alone did not warrant a diagnosis of severe DCS. Mild, transient behavioral changes (agitation or lethargy) were also not considered sufficient for a diagnosis of severe DCS. Once severe DCS was diagnosed, or the hour had passed without evidence of DCS, the animal was euthanized quickly by asphyxiation with He. The chamber was then returned to 1 atm.

Throughout the dive, chamber temperature was thermostatically controlled to values that appeared to be comfortable for the animal (ca. 30°–31°C at 11 atm and 32°–34°C at 22–25 atm).

Analysis of methane release rate: The chamber ventilation rate (115 liter · min^{-1}) was very low (for reasons of economy and safety with H_2) compared to chamber volume at pressure (126,000–145,000 liter). Equilibrium conditions between methane release from the pigs and methane sampling from the chamber would have been reached only after several days. The methane concentration ($[CH_4]$; ppm) in the chamber thus needed mathematical correction to estimate the actual methane release rates from the pigs ($\dot{V}CH_4$) in units of μmol CH_4 · min^{-1}. The method of Bartholomew et al. (11) allows for this correction.

The fractional concentration of CH_4 in chamber gases at equilibrium (X_{eq}) was computed from $[CH_4]$ at two time points during the experiment (X_i and X_{i-1}) separated by a known time interval (Δt) as:

$$x_{eq} = \frac{x_i - x_{i-1}}{1 - e^{\frac{-\dot{v}\Delta t}{V}}} + x_{i-1} \qquad \text{Eq. 2}$$

where $\dot{v}$ is chamber ventilation rate and V is chamber effective volume (chamber physical volume times total pressure). The $\dot{V}CH_4$ was then computed by multiplying X_{eq} by chamber ventilation rate.

The $\dot{V}CH_4$ values were computed for each animal from a mean of five pairs of gas chromatographic measurements of $[CH_4]$, with one member of the pair from the first hour at constant chamber pressure, and the other member from the third hour (Δt = 2 h in each pair).

The lowest quantifiable $[CH_4]$ per chromatographic report for these experiments was 0.5 ppm. Some animals released traces of methane that were too small to accumulate to a concentration of 0.5 ppm after 3 h, and thus are listed as releasing zero methane (Table 1). At the ventilation rates used, animals reported as releasing no methane may have

Table 1: Primary Data for Each Experiment

	Body Mass, kg	H_2, %	c[CH_4], ppm	d[CH_4], ppm	Outcome
22.2 atm					
Total pressure	19.4	92.5 ± 0.1	1.45 ± 0.11	2.88 ± 0.15	0
	18.8	92.4 ± 0.4	0	0	
	18.9	86.1 ± 0.5	1.20 ± 0.14	1.46 ± 0.05	0
	20.0	88.0 ± 0.1	0	0	
	20.0	89,9 ± 0.5	1.63 ± 0.14	2.56 ± 0.17	0
	20.4	89.6 ± 0.2	0.60 ± 0.03	0.85 ± 0.06	0
	21.0	88.3 ± 0.1	2.99 ± 0.16	4.57 ± 0.30	0
	20.7	87.3 ± 0.1	2.53 ± 0.06	3.72 ± 0.23	0
	19,0	87.0 ± 0.4	0.96 ± 0.04	1.52 ± 0.10	0
	19,1	86.0 ± 0.1	1.12 ± 0.08	1.54 ± 0.09	0
	17.9	84.7 ± 0.2	0.74 ± 0.05	0.80 ± 0.05	0
24.0 atm					
Total pressure	19.5	84.3 ± 0.1	1.87 ± 0.12	2.61 ± 0.27	
	22.1	90.9 ± 0.4	1.95 ± 0.11	3.26 ± 0.27	0
	18.2	93.1 ± 0.4	1.78 ± 0.15	2.91 ± 0.11	0
	21.0	88.6 ± 0.1	0.83 ± 0.12	1.83 ± 0.15	0
	21.1	88.8 ± 0.1	1.99 ± 0.09	2.97 ± 0.18	0
	18.0	88.6 ± 0.2	0	 0	0
	18.0	91.6 ± 0.1	0	0	
	19.0	86.6 ± 0.1	0.77 ± 0.08	0.94 ± 0.10	0
	19.4	89.3 ± 0.1	0.78 ± 0.05	0.99 ± 0.03	
25.5 atm					
Total pressure	19.7	88.6 ± 0.0	0.68 ± 0.06	0.69 ± 0.05	0
	17.9	93.1 ± 0.1	0.96 ± 0.08	1.94 ± 0.14	0
	20.2	88.1 ± 0.1	1.89 ± 0.13	3.61 ± 0.62	0
	17.0	91.7 ± 0.1	1.33 ± 0.08	2.15 ± 0.20	0
	20.1	91.7 ± 0.3	1.71 ± 0.13	2.61 ± 0.13	0
	18.6	87.9 ± 0.1	0.51 ± 0.05	0.82 ± 0.05	0
	17.8	92.2 ± 0.1	3.06 ± 0.19	5.22 ± 0.36	0

[a]Including body mass of the pig, mean (±1SEM) H_2 content in percent during the final 36 min at maximal pressure, mean (±1SEM) methane concentration in the chamber during the final hour of compression (c[CH_4]), mean (±1 SEM) methane concentration in the chamber during decompression (d[CH_4]), and outcome with respect to decompression sickness (1 = DCS; 0 = no DCS).

been releasing up to 8 μmol $CH_4 \cdot min^{-1}$.

Statistical analysis: All mean values reported are ±1 standard error. Six factors were hypothesized to be potential modulators of DCS risk in these animals: total chamber pressure, chamber partial pressure of H_2 (PH_2), chamber concentration of H_2 (% H_2), animal body mass, mean chamber [CH_4] during the last hour of compression (c[CH_4]), and mean chamber [CH_4] during decompression (d[CH_4]). We used multivariate logistic regression techniques (12) to determine the probability of DCS, using DCS outcome as the dependent variable and these six factors as independent variables to be tested both sequentially and simultaneously for significance. The log-likelihood ratio test was used to compare models, with significant differences accepted at the $P = 0.05$ level.

RESULTS

A diagnosis of severe DCS was made in 18% (2/11) of animals after exposure to 22.2 atm total pressure, in 33% (3/9) after exposure to 24 atm, and in 0% (0/7) after exposure to 25.5 atm (Table 1).

Four pigs did not release quantifiable amounts of CH_4 during the experiment (Table 1). In all other animals, the c[CH_4] increased over time throughout the hyperbaric exposure (Fig. 1). The c[CH_4] in the last hour at the maximal pressure of the experiment was taken as the index for comparison among animals during the compression phase (Table 1; mean and standard error of 5 chromatographic readings). At the onset of decompression, d[CH_4] typically increased further and then either remained elevated or declined slightly (Fig. 1). Mean d[CH_4] values, which include the period of time the animals subsequently spent at 11 atm, also appear in Table 1 (mean and standard error of 2–7 chromatographic readings).

Of the animals that did not release quantifiable amounts of CH_4, 3 out of 4 had signs of DCS; only 2 out of 23 animals that did release CH_4were diagnosed with

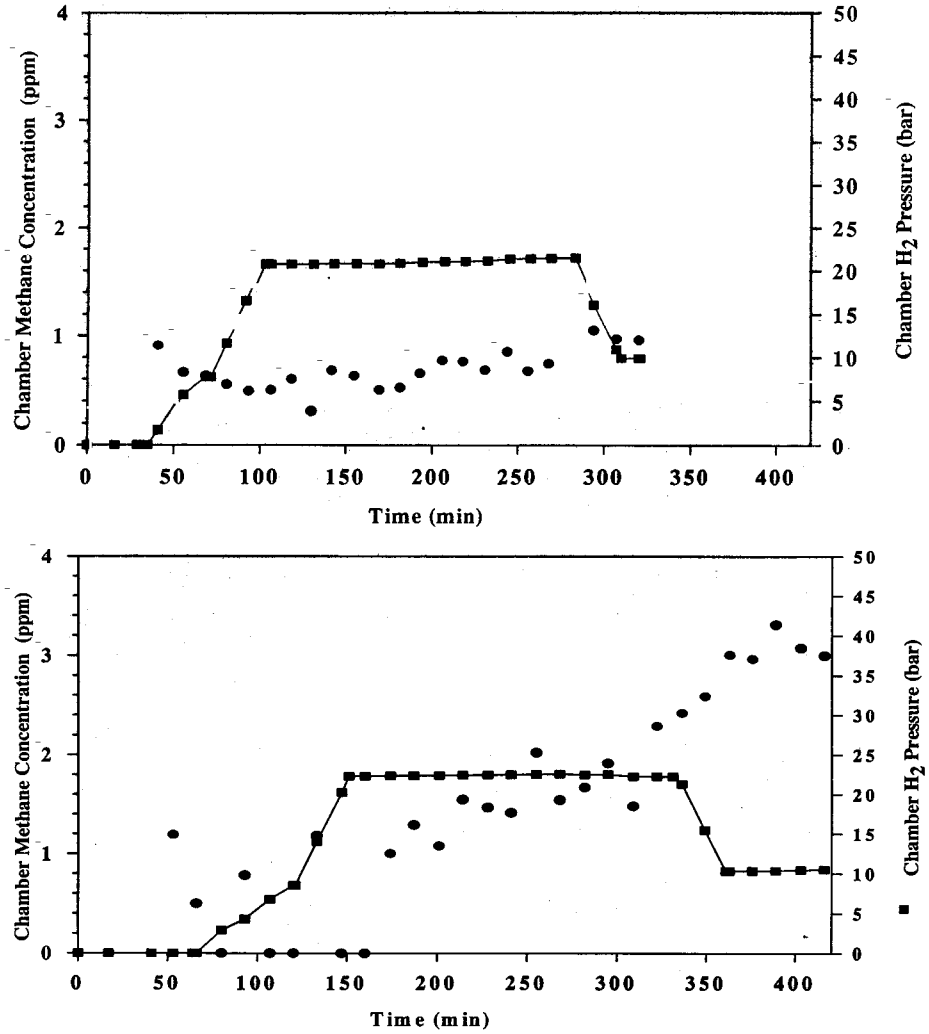

FIG. 1—Sample methane concentration data from two pigs during compression and decompression while breathing H_2. *Top*, pig with subsequent symptoms of severe DCS; *bottom*, pig without subsequent symptoms of severe DCS.

DCS (Table 1). This result is statistically significant ($P < 0.01$, standard two-tailed χ^2 test; $P < 0.05$, two-tailed χ^2 test with Yates correction for small sample sizes).

Chamber pressure, PH_2, % H_2 and the body mass of animals were not contributors to the risk of DCS in this study, as determined by logistic regression (Table 2). From this analysis, we determined that the only variables tested that were significant risk factors for DCS were c[CH_4] and d[CH_4] (Table 2). No combinations of variables were significantly better fits to the data, including a combination of c[CH_4] and d[CH_4]. These two estimates of [CH_4] were highly correlated (98%) with each other. If we used the more conservative measure of taking the 0 ppm values for c[CH_4] and d[CH_4] (Table 1) for the four animals that made less CH_4 than we could quantify reliably (0.5 ppm), and replacing these values with 0.25 ppm (i.e., half the cutoff value), c[CH_4] and d[CH_4] remained significant predictors of DCS (Table 2; LL values changed to -10.718 for c[CH_4] and -10.200 for d[CH_4], β values changed only in their second decimal place, $P < 0.05$). Even going to the very most conservative measure of replacing the 0 ppm values with 0.4 ppm (i.e., 80% of the cutoff detectable value) had minimal impact on the analysis (c[CH_4] trend for significant predictor, $P < 0.10$; d[CH_4] significant predictor, $P < 0.05$).

There was a significantly higher c[CH_4] for animals that did not display signs of DCS (1.40 ± 0.17 ppm CH_4) compared to that of animals diagnosed with DCS (0.53 ± 0.37 ppm CH_4; significantly different, Student's t test, $P < 0.05$; Table 1). Likewise, there was a significantly higher d[CH_4] for animals without DCS (2.22 ± 0.29 ppm CH_4) compared to that of animals with DCS (0.72 ± 0.51 ppm CH_4; significantly different, $P < 0.05$; Table 1).

Table 2: Univariate Logistic Regression Analysis of the Probability of DCS vs. Various Test Variables[a]

Variable	LL	Model P	β ±1 SEM
Constant	-12.937	–	–
Body mass	-12.833	>0.50	–
Pressure	-12.689	>0.40	–
H_2, %	-12.937	>0.90	–
PH_2	-12,736	>0.50	–
c[CH_4]	-10.268	<0.05	-1.82 ± 0.96
d[CH_4]	-9,914	<0.05	-1.23 ± 0.64

[a]Including body mass, chamber pressure, chamber concentration of H_2 (% H_2), chamber partial pressure of H_2 (PH_2), chamber methane concentration during the final hour of compression (c[CH_4]), and chamber methane concentration during decompression (d[CH_4]). Analysis includes the log-likelihoods (LL) of the models with a constant and each of the listed variables tested individually, the probability (P) for each model, and the parameter fit (β) and its standard error for each variable in a significant model.

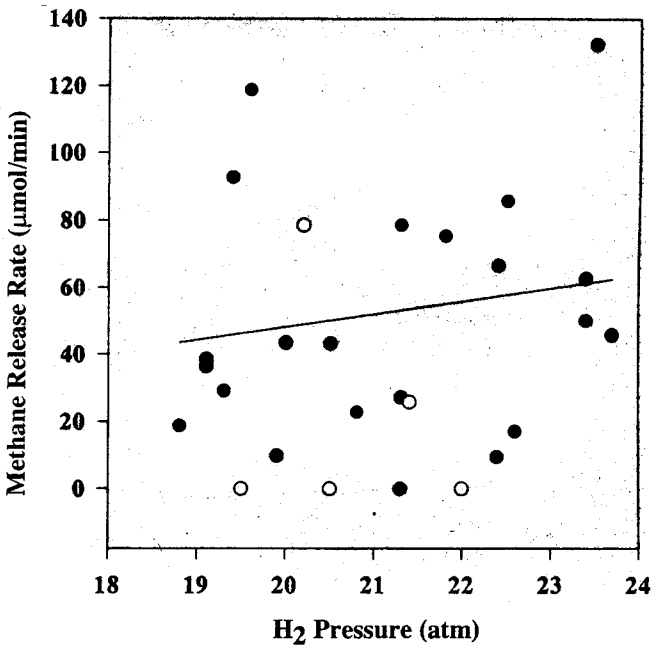

FIG. 2—Mean methane release rate per animal versus maximal H_2 partial pressure for pigs during simulated dives in H_2. *Solid circles*: animals without DCS; *open circles*: animals with DCS. Least squares regression line (not statistically significant) for all non-zero values of methane release rate (Y = -29.04 + 3.86 X, $P = 0.40$, $r^2 = 0.03$; SE of slope = 4.52)

The $\dot{V}CH_4$ values computed for these animals varied widely among individuals, from less than 10 to over 100 μmol $CH_4 \cdot min^{-1}$ (Fig. 2). These values were highly correlated with c[CH_4] (98%) and d[CH_4] (97%), and therefore also predictors of DCS ($P < 0.05$), as expected since $\dot{V}CH_4$ is computed from c[CH_4]. The $\dot{V}CH_4$ values were not significantly correlated ($P > 0.40$) with chamber PH_2 (Fig. 2).

DISCUSSION

Pigs that released readily quantifiable volumes of methane during compression and decompression with H_2 had a lower DCS incidence than pigs that released unquantifiably small volumes of methane (Table 1). Methane release from mammals is strictly an indicator of H_2 metabolism by intestinal microbes, normally using endogenously produced H_2 released as an end product of the metabolism of other intestinal microbes (5). A link between methane release and lower DCS risk is clear. Microbes native to the intestinal flora of these animals eliminated some of the H_2 that would otherwise have been in solution within the tissues of the pigs as a consequence of their breathing hyperbaric H_2. The lower H_2 load in these animals reduced their risk of DCS compared to that of animals that for unknown reasons failed to possess intestinal flora that metabolized H_2 to CH_4. Thus, the concept of H_2 biochemical decompression, that is, using biochemical means to reduce tissue gas load and decrease DCS risk, is supported in this pig model. Only endogenous biochemical processes were exploited to achieve this result. This contrasts with our previous work in which biochemical decompression was demonstrated to reduce DCS risk in a rat model by surgically injecting H_2-metabolizing microbes into their intestines (1). It also contrasts with our original expectations for this study, since we did not anticipate that the activity of native microbes would be sufficient to have a demonstrable effect on DCS risk.

Pigs that released little or no methane while breathing hyperbaric H_2 were seldom encountered, and could not be reliably identified before the dive. We attempted to generate a separate group of methanogen-free animals by treating them for 3–5 days before the dive with heavy doses (1,000 mg twice daily, by mouth) of chloramphenicol. This broad-spectrum antibiotic is used for treating a wide range of bacterial infections, including enteric infections. However, of the six chloramphenicol-treated animals that were exposed to the dive profiles included in the present study, three had accumulated 0.6 ppm CH_4 in the chamber by the end of their 3-h exposure, and the other three were just approaching the minimum reliably quantifiable concentration of CH_4 (0.5 ppm). None of these 6 animals had signs of DCS.

The failure of the antibiotic treatment to eliminate CH_4 release is evidently because the action of chloramphenicol is not specific to methanogens (T.L. Miller, personal communication 1997). An antibiotic specific to these

microbes is not readily identifiable because these Gram-positive anaerobic archae (13) are not in a category of organisms associated with human pathologies (14). None of the chloramphenicol-treated animals were included in this study, since the treatment did not have the intended effect of eliminating the methanogens, and we did not know what other consequences there might have been to these treatments. Certainly, we could not have claimed that chloramphenicol-treated animals had their full native intestinal flora. If we had included an additional three animals releasing 0 $c[CH_4]$ and 0 $d[CH_4]$with 0 incidence of DCS, and three animals releasing detectable $c[CH_4]$ and $d[CH_4]$ with 0 incidence of DCS, this would not have altered our conclusions ($P < 0.05$, standard two-tailed χ^2 test; $P = 0.05$ for logistic regression with $d[CH_4]$; $0.10 > P > 0.05$ for logistic regression with $c[CH_4]$).

These experiments were designed to include a range of total pressures and PH_2 tested, with the expectation that DCS risk would increase with pressure as is conventionally found in DCS studies (15–18), and specifically found for rats in the pressure range explored here (9). The absence of that effect here is notable (Table 1). We attribute this to sufficient tissue H_2 elimination by the native intestinal flora (when present) to reduce the risk of DCS, even for animals at the highest PH_2 we tested.

We attempted to determine if conditions could be reached under which the native intestinal flora of the pigs would be insufficient to protect against DCS. A small group of pigs ($n = 3$) was tested at 30 atm total pressure to determine if a higher PH_2 could overwhelm the native methanogens. However, this approach was abandoned when the pigs at this high pressure were observed to have considerable difficulty walking for 5 min while compressed, and therefore could not be reliably judged for DCS when decompressed. We presumed that the animals were experiencing either joint problems from the lengthy and rapid compression, or high pressure neurologic syndrome. A group of animals ($n = 10$) was also compressed to 24 atm and decompressed at 0.91 atm $\cdot$ min^{-1}, i.e., to one of the same pressures but decompressed at twice the rate used in the experiments reported here. Nine of these 10 animals had DCS (unpublished observation 1999). Thus this more rapid decompression had the intended effect of increasing DCS incidence even in pigs with substantial activities of native methanogens in their intestinal flora (19). Further studies in biochemical decompression using this pig model and including animals receiving intestinal injections of methanogens ultimately included 109 dive exposures, and a total of 53 cases of DCS, with methane release a significant predictor of DCS risk. These studies will be described in detail elsewhere.

Comparing $c[CH_4]$ and $d[CH_4]$ values among animals at the same time points during their respective dives is an unbiased basis of comparison for these animals. However, $c[CH_4]$ and $d[CH_4]$ values were recognized to need mathematical correction to estimate the actual methane release rates from the pigs, given the huge difference between chamber volume and chamber sampling rate (11). The $\dot{V}CH_4$ values (Fig. 2) are extrapolations from 3 h of data to a time point at equilibrium that would have occurred several days later, and are therefore subject to more error than the original $c[CH_4]$ and $d[CH_4]$ data (Table 1 and Fig. 1). However these extrapolations are necessary to relate $c[CH_4]$ and $d[CH_4]$ measurements to the microbial metabolic events occurring inside the animals, which are the true focus of our interests.

If the intestinal methanogens were limited in their metabolism to a large extent by the diffusion of H_2 through the pigs, then there should have been a positive correlation between $\dot{V}CH_4$ and PH_2 (Fig. 2). The absence of such a correlation may be due in part to the uncertainty in our estimations of $\dot{V}CH_4$ and the known variability among individuals in the activity of their native flora of methanogens (5, 20). The relatively small range of PH_2 tested may have been another factor. It may also be erroneous to assume that the methanogens are limited in their metabolism primarily by the rate of H_2 delivery by diffusion as opposed to H_2 delivery by perfusion (1).

Methane release rate estimates are crucial to an estimation of the total volume of H_2 eliminated by the intestinal methanogens, and therefore the link between inert gas load in tissues and DCS risk. The 3-h duration of these experiments is probably sufficient to have brought these pigs near saturation (estimated saturation time of 220 min, A. Fahlman, unpublished observation 1999). If we assume a whole-body value of H_2 solubility of 0.02 ml $H_2 \cdot g^{-1}$ tissues per atm at 37°C (21), a 20-kg pig at 24 atm total pressure and 90% H_2, i.e., 21.6 atm PH_2, should have 8.64 liters of H_2, or 340 mmol H_2 (STP) in solution in its tissues when saturated. The CH_4-producing pigs that were tested at 24 atm released an average of approximately 54 μmol $CH_4 \cdot min^{-1}$ (range 20–80 μmol $CH_4 \cdot min^{-1}$) during the 3 h at maximal pressure (Fig. 2). Thus, the methanogens were responsible for releasing approximately 10 mmol CH_4 during the compression phase of this simulated dive, which required a consumption of 4 times this amount of H_2 *(Eq. 1)*. We can therefore estimate that the methanogens removed 12% (40/340; range 4–17%) of the gas volume predicted to be the total body burden of H_2 in solution in these pigs. While this calculation is fraught with oversimplifications and assumptions (1), it at least allows us to con-

sider the general order of magnitude of gas volumes involved.

We reported previously (1) that removing roughly 5% of the body burden of H_2 from rats decreased their DCS risk by more than 50%. In both rats and pigs, the message appears to be that eliminating relatively small fractions of dissolved gas from animals can have a surprisingly large impact on DCS risk. This observation has also been made in human DCS studies, in which it has been noted that small, conservative measures in computing decompression time can reduce DCS risk by 50% or more (22).

It should be noted that in the present study there was one animal that produced no quantifiable methane and did not display symptoms of DCS, and two animals that produced methane but did have DCS (Table 1). Thus, despite a measure of success in our efforts to make mechanistic predictions of DCS risk, a component of this risk remains approachable strictly as a random event (18).

The finding that methanogens native to the intestinal flora may be protective against DCS during H_2 dives has implications for human health. The potency for causing DCS was found to be 35% greater with H_2 than with He in rats subjected to a variety of time and pressure exposures (9). This result is in keeping with the higher solubility of H_2 than helium in water and lipids, and the general assumption that a greater potential tissue gas volume poses higher DCS risk (9). The presence of methanogens in the intestinal flora of rats is known to be sporadic (23), and the untreated Sprague–Dawley rats used in our previous study did not release detectable volumes of methane while breathing hyperbaric H_2 (1). Consequently, the full DCS potency of H_2 vs. helium is discernable in rats. However, both pigs and humans usually have an intestinal flora containing methanogens, although the activity varies widely between individuals of both species (5, 20). The present research shows that this flora can be protective against DCS. Decompression profiles for human divers using specialty gases such as H_2 are typically established by trial-and-error experience. If a decompression profile for a H_2 dive is selected using mostly divers who possess abundant methanogens in their intestinal flora, such a profile could be dangerous for the occasional H_2 diver who has a methanogen-poor intestinal flora.

This research will continue by demonstrating that pigs in hyperbaric H_2 have further decreases in DCS risk by injecting cultures of methanogens into their intestines (19). The next major step will be to package methanogens in capsules for oral consumption, making high activity H_2 biochemical decompression practical for human divers. The culmination of this research will be an extension from H_2 to N_2 biochemical decompression, making protection against DCS for most divers as simple as swallowing a capsule.

In conclusion, while exposing pigs to hyperbaric H_2, we observed an association between their rate of release of CH_4 and a subsequent reduction in their risk of DCS. We attributed this risk reduction to biochemical processes that metabolized H_2, which were provided by native microbes in their intestines. Confirmation of this observation will require a demonstration of a causal link between increased H_2 metabolizing activity delivered to the intestinal flora and further reductions in DCS risk.

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REFERENCES

1. Kayar SR, Miller TL, Wolin MJ, Aukhert EO, Axley MJ, Kiesow, LA. Decompression sickness risk in rats by microbial removal of dissolved gas. Am J Physiol 1998; 275:R677–R682.
2. Fructus XR. Hydrogen narcosis in man. In: Bauer RW, ed. Hydrogen as a diving gas. Bethesda, MD: Undersea and Hyperbaric Medical Society, 1987:53–56.
3. Abraini JH, Gardette-Chauffour MC, Martinez E, Rostain JC, Lemaire C. Psychophysiological reactions in humans during an open sea dive to 500 m with a hydrogen-helium-oxygen mixture. J Appl Physiol 1994; 76:1113–1118.
4. Fontanari P, Badier M, Guillot C, et al. Changes in maximal performance of inspiratory and skeletal muscles during and after the 7.1-MPa *Hydra 10* record human dive. Eur J Appl Physiol 2000; 81:325–328.
5. Miller TL. Biogenic sources of methane. In: Rogers JE, Whitman WB, eds. Microbial production and consumption of greenhouse gases: methane, nitrogen oxides, and halomethanes. Washington, DC: American Society for Microbiology, 1991: 175–187.

6. Calder III WA. Size, function, and life history. Cambridge, MA: Harvard University Press, 1984.
7. Miller ER, Ullrey DE. The pig as a model for human nutrition. Ann Rev Nutr 1987; 7:361–382.
8. Dromsky, DM, Toner CB, Survanshi S, Fahlman A, Parker E, Weathersby P. The natural history of severe decompression sickness after rapid ascent from air saturation in a porcine model. J Appl Physiol 2000; 89:791–798.
9. Lillo RS, Parker EC, Porter WR. Decompression comparison of helium and hydrogen in rats. J Appl Physiol 1997; 82:892–901.
10. Eichert H, Fischer M. Combustion-related safety aspects of hydrogen in energy applications. Int J Hydr Energy 1986; 11:117–124.
11. Bartholomew GA, Vleck DL, Vleck CM. Instantaneous measurements of oxygen consumption during pre-flight warm-up and post-flight cooling in sphingid and saturniid moths. J Exp Biol 1981; 90:17–32.
12. Hosmer, DW, S Lemeshow. Applied logistic regression. New York: Wiley Press, 1989.
13. Miller TL. Section 25. Archaeobacteria. In: Bergey's manual of systematic bacteriology, vol. 3. Baltimore: Williams & Wilkins, 1989: 2178–2183.
14. Simon GL, Gorbach SL. Intestinal flora and gastrointestinal function. In: Physiology of the gastrointestinal tract, 2nd ed. New York: Raven Press, 1987:1729–1740.
15. Boycott AE, Damant GCC, Haldane JS. The prevention of compressed-air illness. J Hyg (Lond) 1908; 8:342–443.
16. Jacobsen G, Jacobsen JE, Peterson RE,et al. Decompression sickness from saturation diving: a case control study of some diving exposure characteristics. Undersea Hyper Med 1997; 24:73–80.
17. Lillo RS. Effect of N_2-He-O_2 on decompression outcome in rats after variable time-at-depth dives. J Appl Physiol 1988; 64: 2042–2052.
18. Weathersby PK, Homer LD, Flynn ET. On the likelihood of decompression sickness. J Appl Physiol 1984; 57:815–825.
19. Fahlman A, Kayar SR, Lin WC, Whitman WB. Lower risk of decompression sickness in pigs given H_2-metabolizing microbes prior to dives in H_2. FASEB J 1999; 13:A408.
20. Miller TL, Wolin MJ. Enumeration of *Methanobrevibacter smithii* in human feces. Arch Microbiol 1982; 131:12–18.
21. Weathersby PK, Homer LD. Solubility of inert gases in biological fluids and tissues: a review. Undersea Biomed Res 1980; 7:277–296.
22. Weathersby PK, Survanshi SS, Hays JR, MacCallum ME. Statistically based decompression tables III: comparative risk using U.S. Navy, British, and Canadian standard air schedules. Naval Medical Research Institute tech rep, NMRI 86-50, 1986..
23. Maczulak AE, Wolin MJ, Miller TL. Increase in colonic methanogens and total anaerobes in aging rats. Appl Environ Microbiol 1989; 55:2468–2473.