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Effect of inert gas switching at depth on decompression outcome in rats

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LILLO, R. S., AND M. E. MACCALLUM. *Effect of inert gas switching at depth on decompression outcome in rats.* J. Appl. Physiol. 67(4): 1354–1363, 1989.—The present investigation was performed to determine whether inert gas sequencing at depth would affect decompression outcome in rats via the phenomenon of counterdiffusion. Unanesthetized rats (*Rattus norvegicus*) were subjected to simulated dives in either air, 79% He-21% O_2, or 79% Ar-21% O_2; depths ranged from 125 to 175 feet of seawater (4.8–6.3 atmospheres absolute). After 1 h at depth, the dive chamber was vented (with depth held constant) over a 5-min period with the same gas as in the chamber (controls) or one of the other two inert gas-O_2 mixtures. After the gas switch, a 5- to 35-min period was allowed for gas exchange between the animals and chamber atmosphere before rapid decompression to the surface. Substantial changes in the risk of decompression sickness (DCS) were observed after the gas switch because of differences in potencies ($He < N_2 < Ar$) for causing DCS and gas exchange rates ($He > Ar > N_2$) among the three gases. Based on the predicted gas exchange rates, transient increases or decreases in total inert gas pressure would be expected to occur during these experimental conditions. Because of differences in gas potencies, DCS risk may not directly follow the changes in total inert gas pressure. In fact, a decline in predicted DCS risk may occur even as total inert gas pressure is increasing.

gas bubbles; counterdiffusion; hyperbaric; diving; decompression sickness

THE ROLE that different inert gases have in causing decompression sickness (DCS) has been studied in length in both animals and humans (1, 3, 9, 18, 20, 29). These investigations have demonstrated significant differences in inert gas uptake and elimination rates and potency for producing DCS. Because of these differences, the potential appears to exist for using inert gas sequencing to reduce the decompression requirement during operational diving (3, 5, 15). However, it is also believed that the decompression load (i.e., the inert gas that must be eliminated during decompression) could be increased under other gas-sequencing procedures if one gas is taken up by tissues faster than another gas is washed out. Under these circumstances, a transient increase in total inert gas pressure could occur under isobaric conditions via the phenomenon of counterdiffusion (10, 23, 30). In fact, there is some evidence that bubbles can form under such situations (6, 17). Thus it appears important to determine which gas switches might be advantageous and which might be disadvantageous from a decompression safety point of view.

Although various models of inert gas transport in the microcirculation and in tissues have been proposed, the applicability of one model over another in relation to decompression is unclear (5, 12, 23, 30). The effect of switching from one inert gas to another on decompression outcome is, therefore, difficult to predict and undoubtedly depends on the properties of the specific gases as well as the type of DCS (i.e., specific site of bubble development). Unfortunately, only limited measurements have been made of solubility and diffusivity coefficients for many of the tissues that have been implicated in DCS (26), and such measurements for tissues under hyperbaric conditions are even rarer. Performing gas-switching experiments during dives with animals could provide answers regarding decompression risk and gas exchange that are not currently available.

This investigation uses a whole animal model to examine the effect of inert gas switching at depth on decompression outcome. The maximum likelihood technique for fitting mathematical models to binary data is used so that gas exchange rates and gas potencies can be estimated simultaneously. In this manner, the effect of gas switching not only on DCS but also on predicted inert gas pressures in the animal could be examined.

METHODS

Experimental. Male albino rats (*Rattus norvegicus*, Sprague-Dawley strain), weighing 204–313 g, were obtained from a local supplier (Harlan Sprague-Dawley, Indianapolis, IN) and housed at the Naval Medical Research Institute for at least 1 wk before use.

Five animals were placed in a cylindrical cage (64 cm long, 23 cm diam) and compressed together at a rate of 60 feet of seawater (fsw)/min to a pressure of 125, 150, or 175 feet of seawater gauge [fswg; 4.8, 5.5, or 6.3 atmospheres absolute (ATA)] in a Bethlehem model 183610 HP hyperbaric chamber (Bethlehem, PA). The dive mixture was either air, 79% He-21% O_2, or 79% Ar-21% O_2. For air dives, the chamber was closed and then compressed to depth with air. For He-O_2 or Ar-O_2 dives, it was necessary to flush the closed chamber with an appropriate amount of O_2 over a 5-min period before compression to remove all N_2 (20). This purging was then followed by partial compression with O_2 (for the 150 and 175 fsw dives) and then final compression to depth with either He or Ar. For each dive, gas was added in appropriate amounts so that the final mixture was ~20.9% O_2. The following is a summary of the gas

pressures used in the He-O_2 and Ar-O_2 dives: *1*) 125 fswg dives: 33 feet seawater absolute (fswa) O_2 (no compression with O_2) + 125 fswa inert gas; *2*) 150 fswg dives: 38 fswa O_2 (5 fswa compression with O_2) + 145 fswa inert gas *3*) 175 fswg dives: 44 fswa O_2 (11 fswa compression with O_2) + 164 fswa inert gas. Clearly, the procedure for air vs. He-O_2 or Ar-O_2 was quite different with respect to O_2 exposure. This difference was incorporated into the model during analysis, as will be seen later in this paper.

During each phase of compression, the chamber temperature increased. Because each gas was added in units of pressure, the chamber was allowed to cool back to its regulated temperature of 28°C before compression with the next gas was begun. Time-at-depth began the moment chamber temperature had stabilized at 28°C after reaching final depth. Because of the additional time required for cooling during compression, total compression time ranged from ~3 to 6 min, depending on the particular dive. Specific times describing the exact compression profile were generally not recorded during dives. Such detailed recording was not believed necessary for every dive and would have interfered with the actual compression procedures. However, exact times for all compression procedures were recorded for several dives of each type. These data were found to be reproducible for a specific type of dive and were used later during the analysis. Undoubtedly, these average compression profiles do not exactly describe the actual profile of most dives. However, the effect of this error on the final analysis will be shown later to be small.

After 60 min at depth, gas switching was performed. The experimental design called for air and He-O_2 switching combinations and He-O_2 and Ar-O_2 switching combinations. No air and Ar-O_2 combination dives were performed. By convention, a particular switch will be denoted in the form G1/G2, where G1 is the initial dive gas that is displaced during the switching procedure by the switch gas, G2 (e.g., air/He-O_2). Switching was accomplished by venting the chamber (while maintaining depth) with ~7,000 liters of the switch gas mixture over 5 min. Only one depth (175 fswg) was used with the air and He-O_2 dives; this produced levels of DCS incidence suitable for modeling. However, because of large differences between He and Ar in potency for causing DCS in rats, three depths (125, 150, and 175 fswg) were used with the He-O_2 and Ar-O_2 dives. This produced a useful range of DCS incidence levels. An appropriate depth to produce a midrange level of incidence values with Ar-O_2 would have resulted in neglible levels of DCS incidence with He-O_2. Conversely, a depth appropriate for midrange incidence levels with He-O_2 would have caused DCS in nearly all animals with Ar-O_2. Both situations would be undesirable for effective data analysis. Control gas switches were also conducted where the chamber was vented with the same gas as that already in the chamber.

After the 5-min gas-switching procedure, additional time was allowed to elapse with the chamber at depth before rapid decompression to the surface (total decompression <10 s). This postswitch time permitted gas exchange to occur between the animal and the new chamber atmosphere. Postswitch times for the air and He-O_2 dives were chosen to be 5, 15, and 25 min, based on previously reported data on gas exchange rates (19). This provided a range of times believed to be adequate for significant inert gas exchange after the switch. However, results from this first set of dives suggested that a postswitch time >25 min was needed to permit more complete gas exchange in the animal. Therefore, when the second set of dives involving He-O_2 and Ar-O_2 were performed, postswitch times were selected to be 5, 15, and 35 min.

In the case of the air and He-O_2 dives, 6 dives with 5 animals each were conducted for each of the 12 possible combinations of gas switch and postswitch times, resulting in the use of 360 rats for these experiments. For the He-O_2 and Ar-O_2 dives, 3 dives with 5 animals each were conducted for each of the 36 combinations of gas switch, depth of dive, and postswitch time. The only exception to this protocol was with two dives where only four rats were available for each dive. Thus a total of 538 rats were used for the He-O_2 and Ar-O_2 switches. Data from 2 of these 538 rats were omitted because of missing body weights for the animals, resulting in a grand total of 896 rats on which the final modeling is based. The air and He-O_2 dives were performed from April to December 1986; the He-O_2 and Ar-O_2 dives were conducted from June 1987 to January 1988. The experimental design as described is presented in Table 1.

A Beckman F3 paramagnetic oxygen analyzer and a Beckman 865 infrared CO_2 analyzer (Fullerton, CA) were used to analyze chamber atmosphere for O_2 and CO_2 when depth was first reached. The composition was adjusted, if necessary, by adding the appropriate inert gas or O_2. Throughout the exposure, the O_2 and CO_2 levels and chamber pressure were monitored and adjusted at ~10-min intervals, holding O_2 constant at 20.9

TABLE 1. *Experimental design*

Gas Switch (G1/G2)	Depth, fsw	Postswitch Time, min		
		5	15	25
Air and He-O_2 switches				
Air/air	175	X	X	X
Air/He-O_2	175	X	X	X
He-O_2/air	175	X	X	X
He-O_2/He-O_2	175	X	X	X
Gas Switch (G1/G2)	Depth, fsw	Postswitch Time, min		
		5	15	35
He-O_2 and Ar-O_2 switches				
He-O_2/He-O_2	125	Y	Y	Y
	150	Y	Y	Y
	175	Y	Y	Y
He-O_2/Ar-O_2	125	Y	Y	Y
	150	Y	Y	Y
	175	Y	Y	Y
Ar-O_2/He-O_2	125	Y	Y	Y
	150	Y	Y	Y
	175	Y	Y	Y
Ar-O_2/Ar-O_2	125	Y	Y	Y
	150	Y	Y	Y
	175	Y	Y	Y

G1/G2, gas switch, where G1 is initial dive gas that is displaced by switch gas, G2; X, 30 animal dives; Y, 15 animal dives.

± 0.5%. Depth varied by no more than ±2 fsw during the exposure. Soda lime on a tray below the cage kept levels of CO_2 below 0.09% of 1 ATA. Both immediately before the gas-switching procedure was started and ~5 min after the switch had been completed, the chamber atmosphere was analyzed for N_2, He, and Ar, as well as O_2 and CO_2. The 5-min postswitch time was more than adequate to achieve complete mixing of the gases within the chamber. The inert gases were measured using a UTI 100C mass spectrometer (Uthe Technology International, Sunnyvale, CA). In all cases (with the exception of air, which contains 0.9% Ar) measurements of preswitch inert gas concentrations demonstrated that the inert gas being used for the initial part of the dive was always >99.5% of the total inert gas composition, indicating effective O_2 flushing at the start of the dive. Chamber temperature was kept at 28.0 ± 0.5°C throughout the exposure by means of a temperature-controlling unit (Yellow Springs Instrument, Yellow Springs, OH).

Immediately after decompression, the animal cage was removed from the chamber and the animals were observed for 30 min in room air and scored for symptoms of DCS as previously described (20). This length of time has been found to be sufficient for nearly 100% of all DCS cases in rats to become evident. Throughout the dive and the 30-min observation period, animals were exercised by rotating the cage at a perimeter speed of ~3 m/min to ensure that all animals sustained a similar level of activity during and after the dive and to facilitate scoring the animals for signs of DCS. For data analysis, the decompression results were scored as no DCS symptoms, obvious DCS, or death. The category DCS, therefore, included the subset category of death. Only one scorer was involved in these experiments.

After the 30-min postdive period, all surviving animals were killed by inhalation of CO_2. After death, all animals were weighed on a triple-beam balance to the nearest gram.

Data analysis—the model. Mathematical models were fit to the data using the technique of maximum likelihood (8), which is well suited for binary data such as decompression outcome. This technique has been used successfully in the past to analyze both human and small animal dive data (19, 20, 27). Maximum likelihood treats the occurrence of DCS after a dive as a variable event and estimates parameters of a model that predict the probability of DCS. A single dose-response model was formulated to predict the probability of DCS in rats after dives involving gas switches with air, He-O_2, and Ar-O_2. Hypothesis testing using the likelihood ratio statistic (LR) was performed to test significance of parameters. Confidence limits for predicted functions were generated by propagation of error analysis (16).

The dose-response model used for this analysis was the Hill equation

$$\text{probability (DCS)} = \text{dose}^n/(\text{dose}^n + P_{50}^n) \qquad (1)$$

P_{50} represents the dose at which there is a probability of 50% for the occurrence of DCS, and the exponent n is the order of the Hill equation that controls the steepness of the central portion of the sigmoidal curve.

The dose in *Eq. 1* represents a measure of decompression stress and was defined as previously reported (19); this definition corresponded to the traditional idea of total gas supersaturation. In this treatment of dose, all the gas partial pressures in the animal immediately before decompression are added together and the final ambient pressure. 1 ATA or 33 fswa, is subtracted. In the present experiments, the possible gases contributing to the decompression response are N_2, He, Ar, and O_2. However, because O_2 was a fixed percentage (20.9%) of all diving mixtures used, the effect of this gas could not be determined (for a discussion of this topic, see Ref. 19). Thus a contribution of O_2 to the dose was not included in the model. To allow for possible differences in the effect of each gas, each partial pressure is weighted by a relative potency value through multiplication. The resulting equation for dose is as follows

$$\text{dose} = [(RP_{He} \cdot Pti_{He}) + (RP_{N_2} \cdot Pti_{N_2}) + (RP_{Ar} \cdot Pti_{Ar})] - 33.0 \qquad (2)$$

RP_{He}, RP_{N_2}, and RP_{A_2} are relative potency values for He, N_2, and Ar, respectively, and Pti_{He}, Pti_{N_2}, and Pti_{Ar} tissue partial pressures (fswa) in the animal at depth immediately before decompression. This definition of dose implies that initial bubble growth is the primary insult in DCS.

The partial pressures of the genes were reported in fswa. Thus the dose was in units of fswa of inert gas pressure. To estimate relative potencies, one of the potencies had to be fixed so the other two could be calculated relative to it. RP_{N_2} was arbitrarily set at 1.0, so P_{50} would be expressed in terms of P_{N_2} in fswa as done previously (19, 20). The effect of this weighting calculation was to convert He and Ar exposures into equivalent N_2 exposures.

The present experiments require an explicit treatment of gas kinetics during the experimental period. Partial pressures of each gas in the animal were obtained using single-exponential kinetics. After a step change in inert gas pressure at time T0, the tissue pressure of one inert gas (Pti) at time T can be described by the following equation for T > T0

$$Pti = (Pa - Pti_0) \cdot [1 - e^{-(T-T0)/TC}] + Pti_0 \qquad (3)$$

where Pa is the ambient inert pressure after the increase, Pti_0 the initial tissue inert pressure, and TC the time constant affecting the rate of gas uptake or washout. The situation becomes more complicated when calculating tissue pressure during times when ambient inert gas pressure is changing (e.g., during compression or a gas-switching procedure). In the present analysis, tissue inert pressure was estimated using a mathematical technique presented previously by Weathersby et al. (28, p. 42–43). This technique treats a dive as a series of pressure ramps that fully describe the pressure history of the dive. When ambient inert gas pressure changes linearly with time, the solution for tissue pressure is as follows

$$Pti = Pti_0 + [k \cdot (T - T0)] - (k \cdot TC) + [k \cdot TC \cdot e^{-(T-T0)/TC}] \qquad (4)$$

The change in the slope of ambient inert pressure is represented by k. For a series of ramps, *Eq. 4* needs to be summed for each new ramp. This exercise is performed for each inert gas that is being considered, in this case He, N_2, and Ar. For the present analysis, calculations using *Eq. 4* were performed for all inert pressure changes including O_2-venting and gas-switching procedures. All changes were assumed to be linear over either the 5-min O_2-venting or the 5-min gas-switching procedure. Mathematical techniques for treating nonlinear (i.e., exponential) pressure changes using this ramping approach would be extremely complicated and have never been used by the authors of this paper. However, for comparison purposes, the data were also modeled using a much more cumbersome approach involving a large series of exponential washout and uptake functions to account for all pressure changes. This method allowed the gas pressure changes inside the hyperbaric chamber during the switching phase to be modeled as exponential functions.

Thermal equilibrium times allowed during compression phases were also incorporated into the modeling. For analysis, it was assumed that at equilibrium the inert gas partial pressures in the animal became equal to those in the chamber. The same time constant is used for both uptake and washout of each gas: TC_{He}, TC_{N_2}, and TC_{Ar} for He, N_2, and Ar, respectively (for further discussion of this method of estimating inert gas exchange, see Ref. 28).

Because animal weight has a significant effect on decompression outcome, a weight correction term for the dose was included in the model as previously done (19, 20). The correction term was set equal to the animal weight (Wt), normalized to the average weight (260 g) of all animals in the experiments, and raised to an exponent called the weight factor (WtF). Therefore the final expression for dose appeared as follows

$$\text{dose (weight corrected)} = \text{dose} \cdot (\text{Wt}/260)^{\text{Wt}F} \quad (5)$$

In summary, this model *1*) predicts the probability of DCS in rats subjected to hyperbaric exposures of variable time, depth, and gas composition involving gas-switching procedures; *2*) assumes that the decompression response is dependent on pressure changes of the individual gases (He, N_2, and Ar) in the animal; and *3*) is used to estimate the parameters P_{50} and n (governing the location and shape of the dose-response curve), RP_{He}, RP_{N_2}, and RP_{Ar} (relative potencies of the individual gases), WtF (the exponent providing the correction for animal weight), and TC_{He}, TC_{N_2}, and TC_{Ar} (time constants for the 3 inert gases).

RESULTS

Gas-switching effectiveness varied with the specific gas combination, as well as from dive to dive, as is indicated by the He concentration measured in the chamber after the switch (Table 2). Although as complete a gas switch as possible was desired to maximize differences in response due to the switch, the model (as discussed above) used for hypothesis testing does not assume 100% complete switching from the first inert gas to the second inert gas. Instead the model incorporated the actual postswitch inert gas pressures measured in the chamber for each individual dive. These values were then used as variables in estimation of the probability of DCS. In all cases, the level of O_2 of the chamber gas after the switch was 20.9 ± 0.5%.

Data summary. Tables 3 and 4 present decompression results from the air and He-O_2-switching dives performed

TABLE 2. *Effectiveness of gas-switching procedures as measured by postswitch chamber He concentration*

Gas Switch (G1/G2)	Postswitch Time, min	Depth, fsw	%He		
			Minimum	Maximum	Mean
Air and He-O_2 dives					
Air/He-O_2	5	175	89.7	98.7	95.9
	15	175	94.5	98.7	97.1
	25	175	92.3	98.1	96.1
He-O_2/air	5	175	1.2	18.5	8.6
	15	175	2.5	30.0	15.8
	25	175	3.3	21.5	11.8
He-O_2 and Ar-O_2 dives					
He-O_2/Ar-O_2	5	125	0.1	0.7	0.4
	15	125	0.4	4.1	1.8
	35	125	0.5	2.6	1.3
	5	150	0.7	9.8	4.4
	15	150	1.1	11.7	4.6
	35	150	0.9	23.0	8.9
	5	175	0.9	2.4	1.8
	15	175	8.2	10.1	9.0
	35	175	1.8	18.0	7.6
Ar-O_2/He-O_2	5	125	99.7	100.0	99.8
	15	125	100.0	100.0	100.0
	35	125	99.7	100.0	99.8
	5	150	99.7	100.0	99.9
	15	150	99.7	100.0	99.8
	35	150	99.5	99.8	99.7
	5	175	99.7	100.0	99.9
	15	175	96.7	100.0	98.8
	35	175	99.5	100.0	99.7

%He represents %He relative to total inert gas; values were obtained from 3 dives (He-O_2 and Ar-O_2 dives) or 5 dives (air and He-O_2 dives) with 5 rats/dive. Minimum and maximum values represent lowest and highest concentrations observed from the 3 or 5 dives in a group. G1/G2, gas switch, where G1 is initial dive gas that is displaced by switch gas, G2.

TABLE 3. *Experimental data from gas-switching experiments with rats during air and He-O_2 dives at 175 fsw*

Gas Switch (G1/G2)	Post-switch Time, min	Wt, g	DCS Incidence	Death Incidence	Death/DCS
Air/Air	5	253±17	0.77	0.53	0.69
	15	254±16	0.83	0.63	0.76
	25	254±12	0.80	0.53	0.66
Air/He-O_2	5	257±16	0.83	0.53	0.64
	15	261±10	0.80	0.30	0.38
	25	251±25	0.80	0.20	0.25
He-O_2/air	5	257±13	0.13	0.00	0.00
	15	248±12	0.17	0.03	0.18
	25	257±14	0.43	0.23	0.53
He-O_2/He-O_2	5	253±17	0.57	0.07	0.12
	15	251±15	0.43	0.03	0.07
	25	250±17	0.70	0.00	0.00

Values for weight are means ± SD; n = 30 rats/group. G1/G2, gas switch, where G1 is initial dive gas that is displaced by switch gas, G2; DCS, decompression sickness.

TABLE 4. *Experimental data from gas-switching experiments with rats during He-O_2 and Ar-O_2 dives at 125, 150, and 175 fsw*

Gas Switch (G1/G2)	Depth, fsw	Post-switch Time, min	Wt, g	DCS Incidence	Death Incidence	Death/DCS	n
He-O_2/He-O_2	125	5	261±28	0.07	0.00	0.00	15
		15	269±15	0.13	0.00	0.00	15
		35	267±28	0.00	0.00	0.00	15
	150	5	277±21	0.14	0.00	0.00	14
		15	259±15	0.07	0.00	0.00	15
		35	283±18	0.40	0.00	0.00	15
	175	5	268±21	0.47	0.13	0.28	15
		15	273±13	0.73	0.00	0.00	15
		35	270±28	0.33	0.07	0.21	15
He-O_2/Ar-O_2	125	5	251±27	0.07	0.00	0.00	14
		15	256±12	0.47	0.13	0.28	15
		35	282±15	0.60	0.40	0.67	15
	150	5	256±20	0.40	0.33	0.83	15
		15	261±13	0.27	0.20	0.74	15
		35	269±26	0.87	0.73	0.84	15
	175	5	266±27	0.27	0.20	0.74	15
		15	253±29	0.67	0.60	0.90	15
		35	262±20	0.87	0.80	0.92	15
Ar-O_2/He-O_2	125	5	279±19	0.67	0.33	0.49	15
		15	261±21	0.27	0.07	0.26	15
		35	250±15	0.07	0.00	0.00	15
	150	5	264±12	0.67	0.40	0.60	15
		15	263±32	0.73	0.33	0.45	15
		35	266±23	0.07	0.00	0.00	15
	175	5	258±19	0.93	0.73	0.78	15
		15	265±17	0.79	0.36	0.46	14
		35	270±29	0.80	0.27	0.34	15
Ar-O_2/Ar-O_2	125	5	270±18	0.87	0.73	0.84	15
		15	265±17	0.93	0.87	0.94	15
		35	265±24	0.87	0.87	1.00	15
	150	5	272±16	1.00	0.93	0.93	15
		15	262±15	0.87	0.80	0.92	15
		35	265±15	0.87	0.80	0.92	15
	175	5	273±17	1.00	0.93	0.93	15
		15	264±18	1.00	1.00	1.00	15
		35	262±20	0.93	0.93	1.00	14

Values for weight are means ± SD; *n*, no. of rats. See Table 3 footnote for definition of abbreviations.

at 175 fsw, and the He-O_2- and Ar-O_2-switching dives at 125, 150, and 175 fsw. Incidence rates of DCS are expressed as fractions of the total number of animal dives (i.e., number of rats) for each gas switch-time combination.

In the case of the control switches (i.e., air-air, He-O_2–He-O_2, Ar-O_2–Ar-O_2), incidence rates for either DCS or death in most cases do not appear to vary much with increasing postswitch times. However, the uncertainty inherent with binomial samples precludes statistical separation of the few control incidence values that do seem to be different (i.e., the DCS data for the He-O_2–He-O_2 switch in Table 3 or the DCS data for the He-O_2–He-O_2 switch at 150 or 175 fsw in Table 4). Examination of the control data incidence levels suggests that Ar-O_2 is the most potent mixture both in causing DCS and in causing death once DCS has developed (as evidenced by the death-to-DCS ratio). This finding is supported by the apparent change in incidence levels that can be observed after He-O_2–Ar-O_2 and Ar-O_2–He-O_2 switches.

The inherent error associated with the binary data reported in Tables 3 and 4 prevents simple interpretation of the effect of switching from one gas to another on decompression risk. Development of conclusions is further confounded by the range in incidence rates due to the different depths.

The mean weights of all groups presented in Tables 3 and 4 range from 248 to 283 g; the grand mean of all animals was 260 g. Based on 95% confidence limits, there were no significant differences among the groups in weight.

Analysis. P_{50}, relative potencies, Wt*F*, and time constants were estimated separately for DCS and death using the model described by *Eqs. 1–5* (see Table 5). Time constant estimations for the death response had substantially larger standard errors compared with those for DCS. These larger errors undoubtedly reflect the many low death rates, particularly where He-O_2 was involved. Fewer deaths with He undoubtedly caused greater uncertainty in estimating He parameters. This probably accounts for much of the discrepancy of the estimates for time constants and He potency comparing DCS and death. Thus, for purposes of simplification, the death response will be omitted from further discussion.

Estimation of parameters was also performed using a model that assumed the experiment started when depth was first reached and ignored the compression phase of the experiment. With the exception of the time constants, these estimates were essentially identical to those in Table 5. Omitting the compression time increased the time constants slightly: that for He increased ~0.3 min and those for N_2 and Ar increased ~1.0 min. These observations suggest that minor variations that would invariably exist between actual compression profiles and those measured for a few dives and used for modeling should have only small effects on the estimates.

Nearly identical parameter estimates were again obtained when chamber gas pressures during gas switching were modeled as exponential functions. Thus the use of the simplified pressure-ramping model used for analysis did not affect the results, despite the fact that this model

TABLE 5. *Parameters estimated by maximum likelihood model describing decompression outcome after gas-switching procedures*

	DCS	Death
P_{50}, fsw	106.6±5.1	119.9±6.0
n	6.72±0.86	6.33±1.05
RP_{He}	0.835±0.039	0.612±0.060
RP_{N_2}	1.0*	1.0*
RP_{Ar}	1.234±0.044	1.326±0.051
Wt*F*	0.73±0.17	0.70±0.24
TC_{He}, min	8.18±1.51	14.27±6.79
TC_{N_2}, min	16.98±2.54	21.72±6.84
TC_{Ar}, min	13.01±1.60	17.47±4.07

Values are means ± SE unless otherwise indicated. Data used in model prediction are summarized in Tables 3 and 4. Dose is defined by *Eqs. 1–5* in text. Model: probability (DCS or death) = dose^n/(dose^n + P_{50}^n), where dose is dose of decompression stress, P_{50} is depth or pressure that produces 50% incidence, and *n* is exponent controlling slope of response curve. RP_{He}, RP_{N_2}, and RP_{Ar} are relative potencies for He, N_2, and Ar, respectively; TC_{He}, TC_{N_2}, and TC_{Ar} are time constants for gas exchange of He, N_2, and Ar, respectively; Wt*F* is weight factor used to correct dose by weight of animal. * Fixed value.

assumed linear changes in ambient pressure during switching.

Gas potencies. Differences in the relative potencies among the three gases are significant ($P < 0.01$) for the DCS response [as shown by the LR test by fixing the potency for He and Ar at 1.0, the value for N_2 (Table 5)]. Respective potencies for He, N_2, and Ar were ~0.8, 1.0, and 1.2.

Gas time constants. There were significant differences ($P < 0.01$) in the gas exchange time constants among the three gases (Table 5), as shown by LR tests comparing models with one, two, or three time constants. For DCS, the constants for all three gases were statistically different, with the gas exchange rates in the order He > Ar > N_2 (exchange rates are inversely related to the time constants as defined in the model). If equilibrium is defined as four time constants, these experimental results suggest that He equilibrates in ~30 min, Ar in ~50 min, and N_2 in ~70 min. Respective half times [time constant ln(2)] are 6, 9, and 12 min. These time constants, as stated before, define the rate of gas uptake and elimination.

Control gas switches, where the chamber was vented with the same gas as was already in the chamber, interestingly served two functions. In addition to allowing the control response to be measured, the control dives provided decompression outcome data from long-term exposure to constant partial pressures of air, He-O_2, or Ar-O_2. This information was not available from the switching procedures that used two different gases. In retrospect, these data were very important in the analysis, considering the rather long time constants that were estimated. This was demonstrated by fitting the same model to a data set that was identical to the original except that all dives from the control switches were omitted. This resulted in much larger estimations for the time constants with large errors (confidence belts about the time constants in all cases overlapping zero). Because of this, all other parameters were substantially different and also had greater errors.

Weight of animal. Inclusion of a weight correction for the dose resulted in significant improvement in fit ($P < 0.01$), with heavier animals exhibiting a greater probability for DCS [p(DCS)] in these experiments. As previously discussed (19), other biological processes, such as age and length of time that the animals were held in the animal-holding facility, are highly correlated with this "weight effect." These alternative factors cannot be ruled out as contributing to the "weight effect."

Predictive curves. Predictive curves for both p(DCS) and animal gas pressures derived from the DCS parameters in Table 5 are presented in Figs. 1–3. Curves were constructed for many of the experimental switching protocols used so that the actual incidence data could be compared with predictions of the model. For curve generation, rat weight was fixed at 260 g for all curves and postswitch gas concentrations were set equal to the average experimental values reported in Table 2: *1*) air/He-O_2 switch: 5% N_2/95% He; *2*) He-O_2/air switch: 10% He/90% N_2; *3*) He-O_2/Ar-O_2 switch: 5% He/95% Ar; *4*) Ar-O_2/He-O_2 switch: 0% Ar/100% He. The 95% confidence limits of the model predictions are included on the graphs along with the actual incidence data points. One means of evaluating how well the model predictions fit the data would be to test for overlap of the confidence limits of the data with the confidence limits for the predictive curves. Because data confidence limits (not presented here) are rather larger, ranging up to approximately ±30% incidence due to the substantial error associated with binomial samples of this size, there is an overlap in every case between the two respective confidence belts. Thus, there is visual agreement between the model and the data, although the power of this test is weak because of the large errors associated with small subsets of the data.

Figures 1–3 demonstrate that quite dramatic changes can occur in decompression outcome after switching from one inert gas to another. The largest changes in the magnitudes of p(DCS) occur when switching involves He and Ar because these two gases have the most extreme differences in potency for causing DCS. In specific situations [most evident with air and He-O_2 switching (Fig. 1)], there is a clear suggestion that a transient decompression advantage [short-term reduction in p(DCS) when switching from He-O_2 to air] or disadvantage [short-term increase in p(DCS) switching from air to He-O_2] occurs due to differences in the exchange rates of the two inert gases. In the first case (advantage), He washes out of the animal faster than N_2 enters the animal, resulting in a transient decrease (isobaric undersaturation) in total inert gas pressure under isobaric conditions. In the second case (disadvantage), N_2 washes out of the animal slower than He enters the animal, producing a transient increase (isobaric supersaturation) in total inert gas pressure. The predicted changes in gas pressures in the animal that are graphically presented in Fig. 1 clearly illustrate the phenomenon.

However, DCS risk may not directly follow the changes in total inert gas pressure as it does with air and He-O_2 switching. This is the case when switching involves He-O_2 and Ar-O_2 (Figs. 2 and 3). Here, inert gas pressure changes in the animal are predicted to follow a pattern similar to that for air and He-O_2 with transient supersaturation or undersaturation conditions developing, depending on the order of the gas switch. However, the probability function does not directly follow these changes in total inert gas pressure because of the large difference in potency between He and Ar. The contribution that Ar makes to the decompression "dose" (*Eq. 2*) is much larger than that of He because of the greater DCS potency of Ar relative to He. Thus, risk of DCS can be observed to decrease even when total inert gas pressure in the animal is increasing. Conversely, DCS probability can increase when total inert gas pressure in the animal is decreasing.

Comparison with previous dive studies. Two previous rat decompression studies have been reported from this laboratory, one involving saturation exposures using three inert gases (He, N_2, Ar) and a fixed percentage of O_2 (20) and another involving variable time-at-depth dives using two inert gases (He and N_2) and variable O_2 percentage (19). With the exception of the time con-

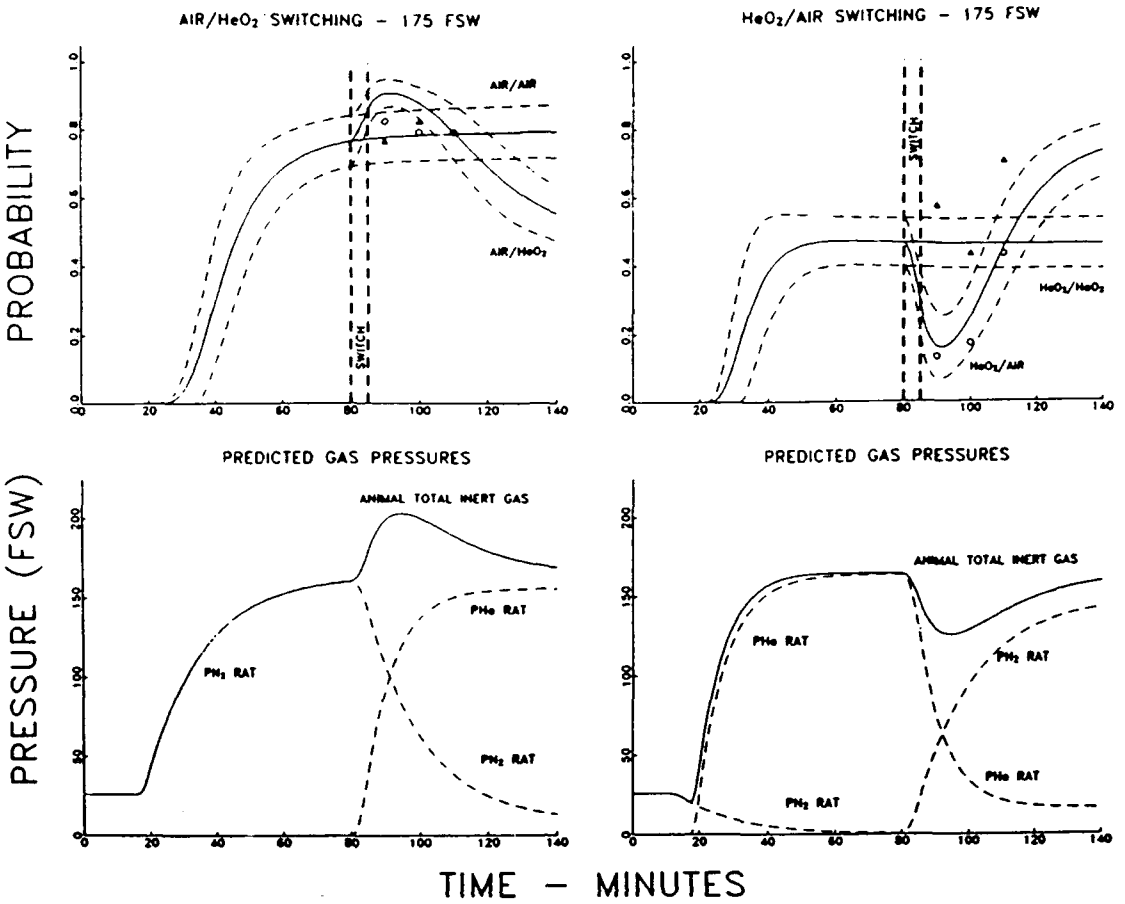

FIG. 1. *Top*: probability of decompression sickness in rats after rapid decompression following switching from air to He-O_2 or from He-O_2 to air at depth (175 fsw). Curves are functions (±2 SD, 95% confidence limits) derived from maximum likelihood model given in Table 5, with weight fixed at 260 g. Each symbol is experimentally observed incidence value, based on 30 animals and on the same units as *Y*-axis. △, Control data where switch gas is the same as initial dive gas; ○, data where switch gas is a different gas. One filled point occurs where △ and ○ overlap. *Bottom*: predicted inert gas pressures in the animal corresponding to graphs at *top*.

stants, there appears to be good agreement between the parameter estimates for the DCS response of these past investigations and those of the present experiments.

DISCUSSION

Theoretical predictions of gas-switching outcome depend heavily on understanding the mechanisms involved in in vivo inert gas exchange. Differences in mass transfer rates of gases into and out of an animal or a human are believed to depend on solubility and diffusion coefficients and partial pressure gradients. Unfortunately, there is a fundamental lack of knowledge regarding such vital aspects as *1*) the relative importance of gaseous diffusion and blood perfusion in gas exchange relative to hyperbaric exposure (12, 13, 23, 30), *2*) the formation and growth of bubbles (2, 24, 25, 31), and *3*) solubility and diffusivity properties of inert gases in tissues under hyperbaric conditions (26). Undoubtedly there are major differences among various tissues in inert gas exchange kinetics. Thus a significant problem in making predictions is that gas-switching effects may vary substantially with the type of tissue that is being considered (*i.e.*, "fatty tissues" vs. "aqueous tissues," intravascular vs. extravascular bubbles). As a result, the potential for increased risk of DCS must be considered dependent on the type of tissue involved (12). Although past experiments suggest that N_2-to-He switching may interfere with normal resolution of bubbles in vitro (22), vascular bubbles (4, 6, 21), and bubbles in adipose tissue (14), the situation for bubbles in other tissues is not clear.

This investigation has demonstrated that gas switching at depth using air, He-O_2, and Ar-O_2 can produce substantial changes in decompression outcome in rats. Significant differences were shown in potencies of the inert gases for causing DCS and time constants for gas exchange. Gas potencies were determined to be in the order $He < N_2 < Ar$ and gas exchange rates in the order $He > Ar > N_2$. Based on these estimates of exchange rates, transient increases (isobaric supersaturation) or decreases (isobaric undersaturation) in total inert gas pressure would be expected to occur during the experimental procedures used here. However, DCS risk may not directly follow the changes in total inert gas pressure but will depend also on the relative differences in potencies of the two gases.

Results from the whole animal decompression model used here suggest that a transient decompression advantage may be obtained by using He-to-N_2 gas sequencing.

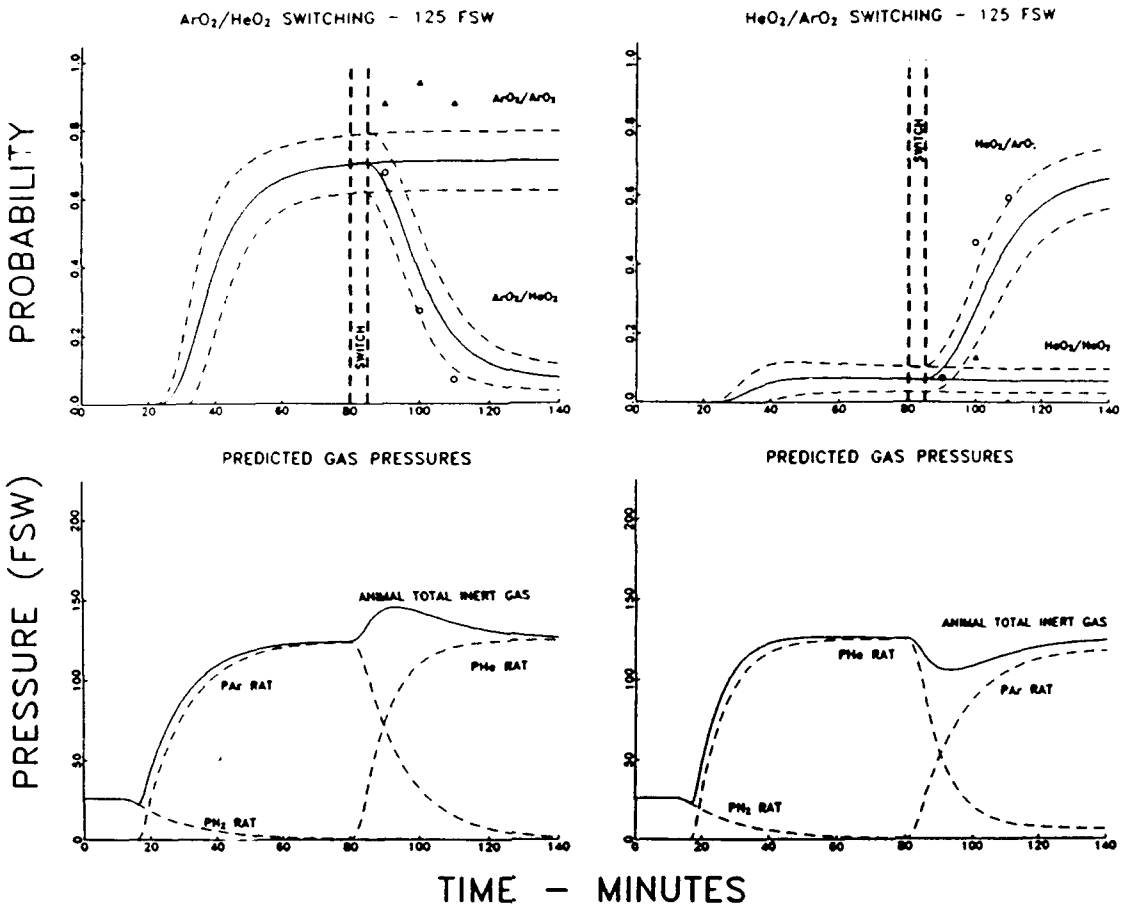

FIG. 2. *Top*: probability of decompression sickness in rats after rapid decompression following switching from Ar-O_2 to He-O_2 or from He-O_2 to Ar-O_2 at depth (125 fsw). Curves are functions (±2 SD, 95% confidence limits) derived from maximum likelihood model given in Table 5, with weight fixed at 260 g. Each symbol is experimentally observed incidence value, based on 14–15 animals and on the same units of *Y*-axis. △, Control data where switch gas is the same as initial dive gas; ○, data where switch gas is a different gas. One filled point occurs where △ and ○ overlap. One control value has a probability value of 0. *Bottom*: predicted inert gas pressures in the animal corresponding to graphs at *top*.

Therefore the reverse switch (N_2 to He) is believed to produce greater risk of DCS over the short term. These observations are in agreement with some previous theoretical arguments involving counterdiffusion (23, 30), experimental work with animals (4, 5, 6, 21), and observations from human decompression trials (3, 15). No such decompression advantage was observed when switching from He to Ar, although some earlier work with humans has suggested that gas-switching procedure might lessen the decompression requirement (15). On the other hand, the analysis presented in this paper did predict the occurrence of transient periods of isobaric supersaturation or undersaturation when He and Ar are used in switching procedures. The apparent paradox between these predictions for He and Ar appears to be due to the differences in potencies for causing DCS that were shown by the present analysis.

Previous theoretical discussions by others have considered only total inert gas pressure changes in examination of the effect of gas switching on potential bubble formation and decompression risk (5, 23, 30). On this basis, results from experimental gas switches have been used to judge the relative importance of processes such as diffusion and perfusion in tissue gas exchange. This contrasts with the present approach of weighting the individual inert gas partial pressures by their relative potency values so that a decline in predicted DCS risk can occur even as total inert gas pressure is increasing. As previously discussed (20), these potency values may be indicative of solubility and/or diffusivity differences and, therefore, may reflect differences in rates of bubble development or total volumes of gas released from solution. Clearly, if such gas potency differences are important, as this study suggests, failure to include them in modeling gas switching may well result in erroneous conclusions.

In marked contrast to the very complicated situation that clearly exists at the tissue level, the present experiments take a simplistic approach to gas loading and elimination and how these processes relate to DCS. Here, the animal is treated as one homogeneous tissue. Gas potencies and gas exchange rates are estimated for the whole animal, and no regard is made for what tissues are actually involved in the DCS response that is being scored. In this case with rats, a very severe type of DCS is being produced with cardiopulmonary and often spinal involvement that frequently results in death. One obvious cause of death is the massive influx of bubbles into

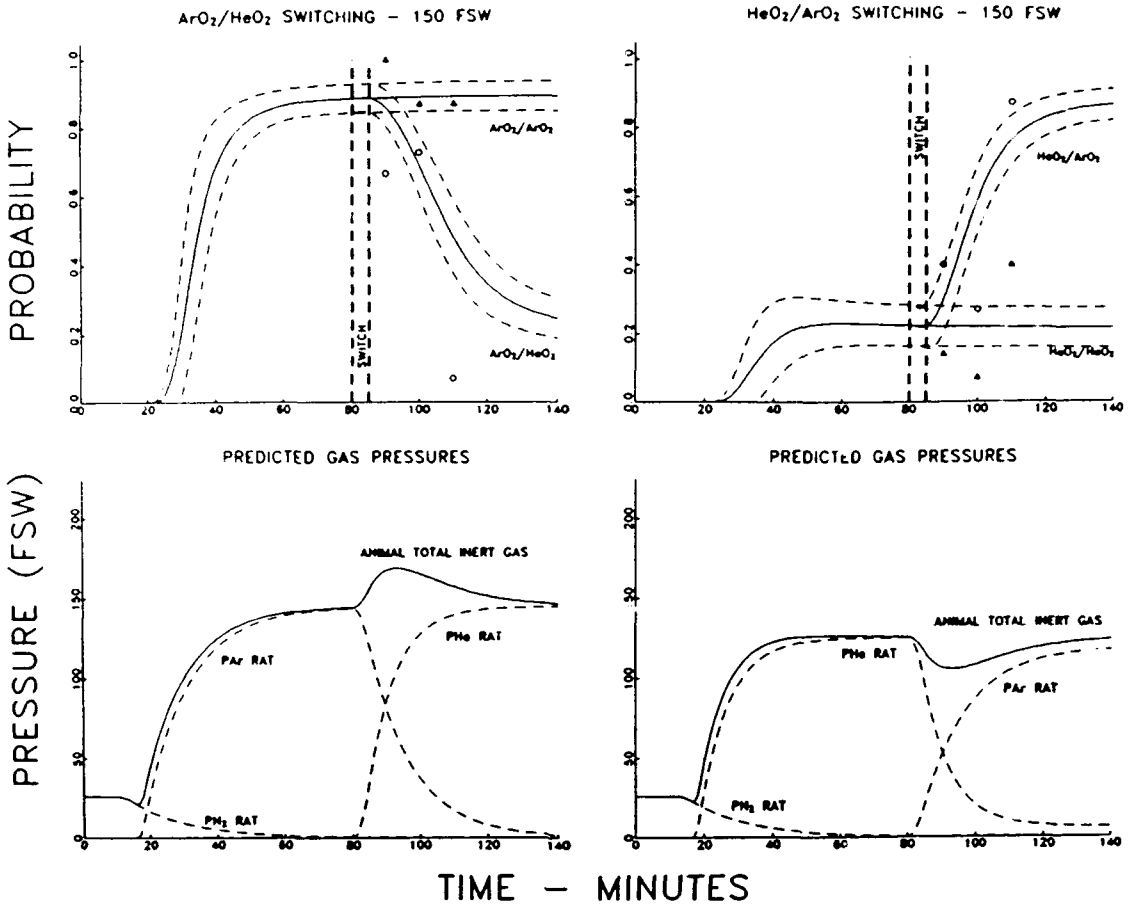

FIG. 3. *Top*: probability of decompression sickness in rats after rapid decompression following switching from Ar-O_2 to He-O_2 or from He-O_2 to Ar-O_2 at depth (150 fsw). Curves are functions (±2 SD, 95% confidence limits) derived from maximum likelihood model given in Table 5, with weight fixed at 260 g. Each symbol is experimentally observed incidence value, based on 14–15 animals and on the same units as *Y*-axis. △, Control data where switch gas is the same as initial dive gas; ○, data where switch gas is a different gas. *Bottom*: predicted inert gas pressures in the animal corresponding to graphs at *top*.

the right side of the heart that can be seen postmortem in most of the animals that die of DCS. Thus the potency and gas exchange rate parameters that are being estimated may be defining the characteristics of whatever tissue systems are responsible for the generation of such intravascular gas. Therefore the basic experimental model used here may limit the applicability of conclusions to human situations. However, this approach has the advantage of being able to examine fundamental decompression questions such as inert gas differences and effects of gas sequencing that would be extremely difficult, if not impossible, to answer by performing human decompression trials.

In a previous study (19) gas uptake rates were reported for He and N_2 also based on rat decompression outcome. Although those exchange rates appear faster than the present values for both uptake and elimination [i.e., He time constant estimated for DCS: 3.09 ± 1.04 vs. the present 8.18 ± 1.51 (SE) min; N_2 time constant: 13.21 ± 1.17 vs. 16.98 ± 2.54 min], the 95% confidence limits do not allow statistical separation. There are, however, a couple of obvious potential errors associated with the time constant estimates from the current experiments. First, the time constants estimated from the current study describe both gas uptake and washout. The assumption made here for simplification is that these rates are equal. However, this assumption may not be correct, and gas washout may occur more slowly than gas uptake (11). Second, the current experiments measured decompression responses over fewer different times (compared with the number of times in Ref. 19), which may limit the accuracy of the time constants.

This investigation has indicated that the potential exists to exploit inert gas sequencing at depth in two ways to achieve a decompression advantage. First, over the short term, differences in the gas exchange rates of two gases may be used to lower the total inert gas pressure in some critical area of the body that is important to DCS development. In this instance, the decompression requirement may be reduced via the phenomenon of counterdiffusion. Whether this reduction occurs appears to be dependent on both inert gas pressures and relative inert gas potencies for causing DCS. Second, over the long term, changes in decompression risk may take place after a gas switch as a result of differences in the relative potency of the gases. Because

total inert gas pressure in the tissue will begin to approach with time the pressure existing before the switch, potency differences will be the predominant factor in determining changes in risk. Performing gas sequencing during decompression would obviously complicate the situation by allowing total pressure to decline as the inert gas makeup changes. Under such conditions, gas transfer between the breathing media and body would be influenced by both the gas switch and drop in absolute exposure pressure. In addition, gas exchange processes may well be different as a result of decompression as suggested by previous experiments (., 11).

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