

# Effect of N<sub>2</sub>-He-O<sub>2</sub> on decompression outcome in rats after variable time-at-depth dives

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LILLO, R. S. *Effect of N<sub>2</sub>-He-O<sub>2</sub> on decompression outcome in rats after variable time-at-depth dives*. J. Appl. Physiol. 64(5): 2042-2052, 1988.—No study of decompression sickness has examined both variable gas mixtures and variable time at depth to the point of statistical significance. This investigation examined the effect of N<sub>2</sub>-He-O<sub>2</sub> on decompression outcome in rats after variable time-at-depth dives. Unanesthetized male albino rats were subjected to one of two series of simulated dives: 1) N<sub>2</sub>-He-O<sub>2</sub> dives (20.9% O<sub>2</sub>) at 175 feet of seawater (fsw) and 2) N<sub>2</sub>-O<sub>2</sub> dives (variable percentage of O<sub>2</sub>; depths from 141 to 207 fsw). Time at depth ranged from 10 to 120 min; rats were then decompressed within 10 s to surface pressure. The probability of decompression sickness (severe bends symptoms or death) was analyzed with a Hill equation model, with parameters for gas potency and equilibrium time for the three gases and weight of the animal. Relative potencies for the three gases were of similar magnitude for bends and statistically different for death in ascending order: O<sub>2</sub> < He < N<sub>2</sub>. Estimated gas uptake rates were different. N<sub>2</sub> took three to four times as long as He to reach full effect; the rate of O<sub>2</sub> appeared to be considerably shorter than that of N<sub>2</sub> or He. The large influence of O<sub>2</sub> on decompression outcome questions the simplistic view that O<sub>2</sub> cannot contribute to the decompression requirement.

decompression sickness; gas bubbles; hyperbaric

ALL VIEWS of decompression sickness (DCS) assume that the original insult is an excess of inert gas somewhere in the body. Unfortunately, little is known about solubility and kinetic properties of gases under high pressure in biologic tissues (16) or how bubbles develop and lead to DCS. Consequently, decompression procedures have been developed based on assumptions and guesses rather than on proven mechanisms of gas interactions with the body. Such an empirical approach involves trial-and-error testing, modification, and retesting of candidate decompression procedures before acceptance. However, results from such methodology have had little success in optimizing the safety or time of decompression for dives other than those with which much practical experience exists. Ideally a scientific examination of the mechanisms involved in gas exchange and in the development of DCS would provide a more efficient approach to the question: how much decompression is needed after a specific dive profile?

The role that O<sub>2</sub> plays in DCS is unclear. Most calculations designed to estimate the decompression requirement ignore O<sub>2</sub> and include only the inert gases such as N<sub>2</sub> and He. The premise behind the equivalent air depth (EAD) concept (2) is that O<sub>2</sub> is metabolized too quickly

to be an important factor in bubble development and growth. EAD equates the decompression requirement of any N<sub>2</sub>-O<sub>2</sub> dive to that of an air dive with identical PN<sub>2</sub>. However, results from a number of animal experiments suggest that O<sub>2</sub> may have a significant influence on decompression outcome. Rashbass and Eaton (15) estimated from work with rats that one-fourth to one-third of the O<sub>2</sub> pressure should be added to the total inert gas pressure for decompression calculations. Donald (6) also concluded from studies with goats that O<sub>2</sub> supersaturation constituted a real danger to safe decompression and questioned the view that metabolism would prevent such supersaturation for any amount of time. Based on hyperbaric work with rats, Berghage and McCracken (3) found that additions of O<sub>2</sub> to the exposure mixture reduced the incidence of DCS under certain conditions, although not in the systematic manner as would be predicted by the EAD concept.

It is not clear how mixtures of different inert gases, such as N<sub>2</sub> and He, combine to affect the decompression requirement. A number of past studies have addressed this issue (1, 4, 8, 11, 12, 19), but disagreement remains concerning whether use of inert gas mixtures can be beneficial in reducing the decompression load. These studies have dealt with both diving on a fixed inert gas mixture and sequencing single inert gas mixtures during a single dive to reduce the time that is needed for safe decompression. Decompression stress could be minimized because of reduced DCS potency of particular mixtures or reduced total inert gas tension brought about by gas switching at depth or during decompression (4, 20, 21). In these cases, both the potency for causing DCS and gas exchange rates would be important factors affecting decompression requirements.

Review of past studies reveals severe limitations on sensitivity due to the nature of binomial distributions. In measuring the incidence of "bends" from a population of divers or animals, typical binomial samples lead to large confidence limits on the measurements. For example, a study resulting in 4 cases of DCS out of 50 animals cannot be statistically separated from another group of 10 cases out of 50 animals, despite the apparent difference of 8 vs. 20%. Recently the technique of maximum likelihood (7) has been adapted to allow the expression of the probability of DCS occurring as a dose-response function that includes variables such as composition of the breathing gas and pressure profile (17). Rather than assuming a precise separation between safe and unsafe dives, which is normally done in decompression

sion table development, probabilistic models consider a given dive as having a finite risk of DCS. Current success with maximum likelihood modeling suggests that with proper experimental designs such a technique could be of considerable value in improving the precision of data analysis. Error associated with hypothesis testing in maximum likelihood is considerably reduced by working with dose-response curves made up of multiple depth and mixture points rather than comparing a few experimental groups. Models can be formulated to account for many factors (such as gas potencies, gas exchange rates, and animal weight), which may have strong influence on the response.

The approach employed in this study is to use the decompression response of a whole animal as a means of quantifying the outcome of decompression. Rats were chosen as the experimental animal because of their uniformity and availability and because of extensive experience with these animals in decompression research. The end point of debilitating DCS or death in these small animals may preclude precise applicability of conclusions to human diving situations. However, these studies allow relatively easy manipulation of the amount of decompression stress.

Several years of experience with the technique of maximum likelihood analysis have demonstrated the need for the use of hundreds of animals to solve simple problems such as the effect of different gases on decompression outcome. The need for such large numbers of animals also makes rats preferable to larger species, despite their lesser similarity to humans. An earlier study (13) attempted to minimize kinetic considerations by using saturation dives followed by very rapid decompression. Differences in the DCS potency of He, N<sub>2</sub>, and Ar were established, and the importance of animal weight in the prediction of decompression outcome was shown. This study is an extension into variable time-at-depth dives with N<sub>2</sub>-He-O<sub>2</sub> designed to look at the effect of two main factors on decompression outcome: 1) inert gas uptake rates and 2) potency of O<sub>2</sub> relative to that of He and N<sub>2</sub>. As before, very rapid decompression was used to reduce the role of gas elimination during decompression so that this factor could be ignored in analysis of data.

## METHODS

### Experimental

Male albino rats (*Rattus norvegicus*, Sprague-Dawley strain) weighing 184–331 g were obtained from a local supplier and housed locally at least 1 wk before use.

**Series I: N<sub>2</sub>-He-O<sub>2</sub> dives (79.1% inert gas = 20.9% O<sub>2</sub>) at 175 fswg (feet of seawater gauge).** These experiments were designed to compare the potency and gas uptake rates of N<sub>2</sub> and He related to decompression outcome. Five animals were placed in a cylindrical cage (64 cm length, 23 cm diam) and compressed together at a rate of 60 fsw/min to a pressure of 175 fswg in a hyperbaric chamber (model 183610 HP, Bethlehem, Bethlehem, PA). They were held at depth for a period of 10–120 min using 10 times at depth: 10, 15, 20, 25, 30, 40, 45, 60, 90, and 120 min. On completing the specified time at depth, the chamber was decompressed to the surface within 10 s. Such a rapid rate of decompression has not been found

to cause pulmonary barotrauma in small animals such as rats that have relatively high ventilatory rates. Animals can be rapidly decompressed after being at depth for only a few minutes on air (incurring little decompression requirement) without any observed ill effects. Occurrence of gross symptoms of DCS (1) and detection of venous gas emboli by Doppler (14) in rapidly decompressed rats have been observed to follow typical dose-response characteristics with incidence rates increasing from 0 to 100% as decompression magnitude and/or time at depth increases.

Four gas mixtures of 79.1% inert gas-20.9% O<sub>2</sub> were used. The four inert gas fractions were as follows: 1) 100% N<sub>2</sub>, 2) 67% N<sub>2</sub>-33% He, 3) 33% N<sub>2</sub>-67% He, and 4) 100% He. Five or six dives with five rats each were conducted at each time-mixture combination in random sequence. The gas mixtures were made by serially adding air or pure O<sub>2</sub>, N<sub>2</sub>, or He to the chamber during compression with the precise amounts depending on the intended composition. In cases where no N<sub>2</sub> was to be present, the chamber was flushed with a predetermined amount of O<sub>2</sub> over a 5-min period before compression (13). The composition of the chamber atmosphere (inert gases, O<sub>2</sub>, and CO<sub>2</sub>) was checked when depth was first reached with a mass spectrometer (UTI 100C, Uthe Technology International, Sunnyvale, CA), a Beckman F3 paramagnetic O<sub>2</sub> analyzer (Beckman Instruments, Fullerton, CA), and a Beckman 865 infrared CO<sub>2</sub> analyzer. If necessary, the composition was adjusted. Throughout the exposure, the gas composition was monitored and adjusted at ~10-min intervals so that all inert gas percentages were maintained within ±1% of intended composition and the percentage of O<sub>2</sub> was held constant at 20.9 ± 0.4%. Soda lime on a tray below the cage absorbed CO<sub>2</sub>. Levels of CO<sub>2</sub> were never observed to rise >0.08%. 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).

Time at depth was defined as starting when depth was first reached and temperature had stabilized at 28°C. Because heating occurred during the compression phase, temperature stabilization generally took 10–30 s after depth was first reached. In addition, several different gases were often used sequentially to compress the chamber. 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. This again required additional time. This procedure resulted in total compression times from ~3.5 to 5.5 min. The 33% N<sub>2</sub>-67% He-20.9% O<sub>2</sub> required the longest total compression time and involved three separate compression phases (starting with the chamber filled with air at the surface): 1) 1.7 min with O<sub>2</sub>, followed by 2) 1.7 min with N<sub>2</sub>, followed by 3) 2 min with He. All times were approximate, and exact times varied somewhat from dive to dive.

Immediately after decompression, the animal cage was removed from the chamber and exposed to room air. The rats were then observed for 30 min for signs of DCS as previously described (13). This length of time has been found to be sufficient for nearly 100% of all DCS cases in rats to become evident (i.e., 98.5% of all DCS cases

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were scored as occurring within 9 min of decompression in the previous saturation dive experiments). Throughout the dive and the 30-min postdive period, animals were exercised by rotating the cage at a perimeter speed of 3 m/min to ensure that all animals sustained a constant level of activity and to facilitate scoring the animals for signs of DCS. For data analysis, the decompression results were scored as follows: 1) no symptoms, 2) obvious bends including death (i.e., motor problems related to walking, rolling in cage, paralysis, or death), or 3) death. The category bends therefore included the subset category of death. Only one scorer was involved in this series of experiments.

After the 30-min postdive period, all surviving animals were killed by inhalation of CO<sub>2</sub>. After death, all animals were weighed on a triple-beam balance to the nearest gram. These experiments were performed over a 9-mo period (July 1983–March 1984). A total of 1,064 rats were used for the dives.

**Series II: N<sub>2</sub>-O<sub>2</sub> dives (variable percentage of O<sub>2</sub> and depth).** These experiments were conducted to determine whether O<sub>2</sub> is indeed innocuous and if its presence may be ignored in decompression. The experiments were conducted in a manner similar to those in *series I*. Seven times at depth were used (10, 20, 30, 45, 60, 90, and 120 min) with each of five different gas mixtures. The gas mixtures contained 141 fswa (feet of seawater absolute) N<sub>2</sub> + one of the following: 1) 33 fswa O<sub>2</sub> = depth of 141 fswg, 2) 66 fswa O<sub>2</sub> = depth of 174 fswg, 3) 99 fswa O<sub>2</sub> = depth of 207 fswg, 4) 33 fswa O<sub>2</sub> + 33 fswa N<sub>2</sub> = depth of 174 fswg, or 5) 33 fswa O<sub>2</sub> + 66 fswa N<sub>2</sub> = depth of 207 fswg. Three or five dives with five rats each were conducted at each time-mixture combination again in random sequence. Gas mixtures were made and adjusted as in *series I*. The compression times with these mixtures ranged from ~4 to 5 min.

These experiments were performed over a 6-mo period (August 1984–January 1985). A total of 727 rats were used for these dives. Because of personnel turnover a different scorer was employed in this experimental series than in *series I*. The criteria for scoring were identical.

#### Data Analysis—the Model

The maximum likelihood technique was used to fit mathematical models to the data (7); its advantage and suitability for binary data such as decompression outcome has been addressed earlier (13, 17). Briefly, maximum likelihood treats the occurrence of DCS after a dive as a random event and estimates parameters of a model that predicts the probability of DCS. A single Hill equation dose-response model was formulated to predict the probability of DCS

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

$P_{50}$  represents the dose at which there is a 50% probability for the occurrence of DCS, and  $n$  represents the order of the Hill equation that controls the steepness of the central portion of the sigmoidal curve. The dose includes terms for each of the gases as well as an animal weight term as described below. The Hill equation describes well dose-response relationships, although alternative models have worked well in other studies (17).

The dose in *Eq. 1* represents a measure of decompression stress and needs to be defined. In previous work with the three gases (He, N<sub>2</sub>, and Ar), the dose that provided an excellent description of the data used raw absolute pressures with no pressure subtraction. Another definition, which corresponds to the traditional idea of total gas supersaturation, would be to add all gas partial pressures together and then subtract the final ambient pressure, 1 atmosphere absolute (ATA) or 33 fsw. To make allowance for possible differences in the effect of each gas, each partial pressure is weighted by a relative potency value through multiplication

$$\text{dose} = [(\text{RPHe} \cdot \text{PHe}_p) + (\text{RPN}_2 \cdot \text{PN}_{2p})] + (\text{RPO}_2 \cdot \text{PO}_{2p}) - 33.0 \quad (2)$$

RPHe, RPN<sub>2</sub>, and RPO<sub>2</sub> are relative potency values for He, N<sub>2</sub>, and O<sub>2</sub>, respectively; PN<sub>2p</sub>, PHe<sub>p</sub>, and PO<sub>2p</sub> are the partial pressures at depth immediately before decompression (predecompression pressures). This was the definition of dose that is used here and would apply to the situation where initial bubble growth is the primary insult in DCS.

Previous work with saturation dives did not account for the changes in the gas partial pressures during the dive and decompression. The effect of dive duration in the present experiments required an explicit treatment of gas kinetics. Each gas partial pressure at depth (predecompression pressure) was defined by an exponential function incorporating two variables, time and chamber ambient partial pressure, and one estimated parameter, the time constant for gas movement. Traditionally a one-exponential function is used for gas kinetics even though real tissues need a more complex model (18). Thus the predecompression pressures were expressed as

$$\text{PN}_{2p} = e^{(-\text{time}/\text{TCN}_2)} \cdot (33.0 \cdot 0.791) + [1.0 - e^{(-\text{time}/\text{TCN}_2)}] \cdot \text{PN}_2 \quad (3)$$

$$\text{PHe}_p = (1.0 - e^{(-\text{time}/\text{TCH}_e)}) \cdot \text{PHe} \quad (4)$$

$$\text{PO}_{2p} = (1.0 - e^{(-\text{time}/\text{TCO}_2)}) \cdot \text{PO}_2 \quad (5)$$

TCN<sub>2</sub>, TCH<sub>e</sub>, and TCO<sub>2</sub> are the time constants for gas entry (and exit); PN<sub>2</sub>, PHe, and PO<sub>2</sub> are the ambient (absolute) partial pressures inside the hyperbaric chamber. The term for N<sub>2</sub> accounts for the animal being saturated on 1 ATA of air at the start of the dive and is defined to be the sum of 1) the original N<sub>2</sub> in the animal equal to the PN<sub>2</sub> of air that washes out during the dive and 2) the N<sub>2</sub> picked up by the animal during hyperbaric exposure. The original O<sub>2</sub> in the animal before the dive was ignored. With sufficient time at depth, this model assumes that the gas tensions within the animal will become equal to ambient pressures of N<sub>2</sub>, He, and O<sub>2</sub>.

Because animal weight has a significant effect, a weight correction term for the dose of decompression stress was included in the model as before (13). This correction term was set equal to the animal weight (Wt), normalized to the average weight (260 g), and raised to a power called the weight factor (Wt<sup>F</sup>). The mean weight of all animals of both series of experiments was 258 g. Therefore the expression for dose appeared as follows

$$\text{dose (Wt corrected)} = \text{dose} \cdot (\text{Wt}/260)^{\text{Wt}^F} \quad (6)$$

where  $WtF$  is the exponent governing the correction for weight. This final model, defined by Eqs. 1-6, is used in most of the following analyses.

As in previous work (13), the partial pressures of the gases were reported in feet of seawater absolute. Thus the dose was in units of feet of seawater absolute of inert gas pressure. To estimate relative potencies, one of the potencies had to be fixed so the other two potencies could be calculated relative to it.  $RPN_2$  was arbitrarily set at one, so that  $P_{50}$  would be expressed in terms of  $PN_2$  in feet of seawater absolute as in previous work (13). The effect of this weighting calculation was to convert He and  $O_2$  exposures into equivalent  $N_2$  exposures.

In summary, this model (1) predicts the probability of DCS (bends or death) in rats subjected to a variable time, depth, and gas composition exposure; 2) includes plausible physiological dependence on pressure changes of the individual gases, He,  $N_2$ , and  $O_2$ , and animal weight; and 3) is used to obtain estimates of the parameters,  $P_{50}$  and  $n$  (governing the location and shape of the dose-response curve),  $RPHe$ ,  $RPN_2$ , and  $RPO_2$ ,  $WtF$ , and  $TCHe$ ,  $TCN_2$ , and  $TCO_2$ .

#### Parameter Estimation and Hypothesis Testing by Maximum Likelihood

Once the above model was selected, maximum likelihood was used to apply the model to the data and estimate parameters so that experimental data and model predictions are in closest agreement. This procedure and hypothesis testing have been discussed previously (7, 13, 17). Hypothesis testing was accomplished by calculation of a likelihood ratio statistic ( $LR$ ). As before, propagation of error analysis (10) was used to generate confidence limits for the predictive curves.

## RESULTS

### Data Summary

Table 1 presents decompression results from the series I  $N_2$ -He- $O_2$  dives at 175 fsw. The incidence rates of DCS are expressed as fractions of the total number of 25-30 animal dives for each time-mixture combination. The incidence of bends and death generally appears to increase with time for those mixtures containing  $>50\%$   $N_2$  as the inert gas fraction. For mixtures with  $<50\%$   $N_2$  as the inert gas fraction, the pattern of decompression response vs. time is less apparent. If we look only at the data from 120 min, mixtures predominantly consisting of  $N_2$  show a greater tendency to produce death (higher death/bends ratio) after initial DCS symptoms than mixtures predominantly consisting of He. Note the variability between adjacent groups in Table 1, but also realize that binomial uncertainty cannot separate incidence values that appear to be different (i.e., 0.20 vs. 0.36 based on 25 animals).

Table 2 presents decompression results from the series II  $N_2$ - $O_2$  dives at variable depth. Incidence rates in this series are based on a total of 14-25 animal dives for each time-mixture combination. Generally, all five mixture groups reveal increasing bends and death incidence with time. Increasing  $PO_2$  as well as  $PN_2$  appear to result in higher levels of DCS.

The mean weight of all time-mixture groups in series I and II ranged from 240 to 283 g. Based on 95% confidence limits, there were no significant differences in weight among the groups.

### Analysis

During analysis, it was impossible to obtain an accurate estimate of  $TCO_2$ , i.e., one with a small SE. For bends, the estimated time constant was a small number ( $<1$  min) with a much larger SE. A better fit for bends (as judged by the  $LR$  test) was achieved when  $TCO_2$  was fixed at 0.000001, and this was done for all analysis reported here. This effectively eliminated  $TCO_2$  from the model and set the predecompression  $PO_{2P}$  and  $PO_{2P}$  equal to the  $PO_2$  of the chamber atmosphere. The effect due to  $O_2$  was therefore equivalent to being instantaneous.

$P_{50}$ ,  $n$ , relative potencies,  $WtF$ , and time constants were estimated using the weight-corrected dose definition defined by Eqs. 1-6, separately for the probability of bends and the probability of death (Table 3). By comparison of parameters for bends vs. death, all overlap in their two SE bounds in Table 3 except for the  $n$  exponent and  $RPO_2$ . All dives from series I and II were used to form the data base.  $LR$  tests for combining data sets (series I and II) revealed a nonsignificant ( $P > 0.05$ ) difference between model fits for each series separately compared with a pooled analysis. These findings support combining data from the two series. Other alternative dose definitions, using either raw pressures with no pressure subtraction or slight modifications of Eq. 2, fit the data no better than the definition used and generated similar parameter estimates.

### Gas Potencies

Differences in the relative potencies among the three gases are significant ( $P < 0.01$ ) for the death response, but not for bends [as shown by the  $LR$  test by fixing all 3 potencies at 1.0 for both bends and death (Table 3)]. These conclusions are also supported by examination of the 95% confidence limits of the potencies. Respective death potencies for  $O_2$ , He, and  $N_2$  were  $\sim 0.4$ , 0.9, and 1.0. Surprisingly the relative potency of  $O_2$  is rather substantial for both responses, although considerably larger for bends. Indeed, for bends, the upper confidence limit would overlap the potencies of He and  $N_2$ , whereas the lower limit would still leave  $O_2$  75% as potent as  $N_2$ . Because  $RPO_2$  is less for death, it ensures that a rat is less likely to die than to be bent despite similar dose-response curves otherwise.

### Gas TC's

Differences in the  $TCHe$  and  $TCN_2$  were significant for both bends and death (as shown by an  $LR$  test comparing individual time constants for the two gases with one time constant for both gases). If equilibrium is considered as four time constants,  $N_2$  has equilibrated by  $\sim 50$  min and He by 12 min (bends) or 19 min (death). The half times [time constant  $\cdot \ln(2)$ ] are 9 min for  $N_2$  and 2-3 min for He. As stated previously,  $TCO_2$  was fixed. A further set of  $LR$  tests established that  $TCO_2$  was significantly below the lowest possible  $TCN_2$  (lower

TABLE 1. *Experimental data from variable time-at-depth dives with rats (series I)*

| Inert Gas Composition      | Time, min | Wt, g  | Bends Incidence | Death Incidence | Death-to-Bends Ratio | N  |
|----------------------------|-----------|--------|-----------------|-----------------|----------------------|----|
| 100% N <sub>2</sub>        | 10        | 259±18 | 0.04            | 0.00            | 0.00                 | 25 |
|                            | 15        | 244±17 | 0.00            | 0.00            | U                    | 25 |
|                            | 20        | 262±22 | 0.30            | 0.03            | 0.11                 | 30 |
|                            | 25        | 260±22 | 0.30            | 0.10            | 0.33                 | 30 |
|                            | 30        | 260±25 | 0.23            | 0.07            | 0.29                 | 30 |
|                            | 40        | 261±15 | 0.60            | 0.36            | 0.60                 | 25 |
|                            | 45        | 258±13 | 0.48            | 0.28            | 0.58                 | 25 |
|                            | 60        | 249±19 | 0.60            | 0.44            | 0.73                 | 25 |
|                            | 90        | 260±12 | 0.68            | 0.44            | 0.65                 | 25 |
|                            | 120       | 266±21 | 0.80            | 0.63            | 0.79                 | 30 |
| 67% N <sub>2</sub> -33% He | 10        | 257±14 | 0.12            | 0.00            | 0.00                 | 25 |
|                            | 15        | 253±16 | 0.20            | 0.04            | 0.20                 | 25 |
|                            | 20        | 267±21 | 0.57            | 0.33            | 0.59                 | 30 |
|                            | 25        | 254±13 | 0.36            | 0.08            | 0.22                 | 25 |
|                            | 30        | 255±15 | 0.52            | 0.40            | 0.77                 | 25 |
|                            | 40        | 247±17 | 0.56            | 0.28            | 0.50                 | 25 |
|                            | 45        | 260±14 | 0.64            | 0.40            | 0.63                 | 25 |
|                            | 60        | 263±21 | 0.55            | 0.24            | 0.44                 | 29 |
|                            | 90        | 252±15 | 0.52            | 0.32            | 0.62                 | 25 |
|                            | 120       | 255±23 | 0.77            | 0.63            | 0.83                 | 30 |
| 33% N <sub>2</sub> -67% He | 10        | 258±17 | 0.64            | 0.08            | 0.13                 | 25 |
|                            | 15        | 256±17 | 0.48            | 0.20            | 0.42                 | 25 |
|                            | 20        | 251±13 | 0.60            | 0.28            | 0.47                 | 25 |
|                            | 25        | 253±13 | 0.36            | 0.24            | 0.67                 | 25 |
|                            | 30        | 261±20 | 0.68            | 0.44            | 0.65                 | 25 |
|                            | 40        | 257±20 | 0.80            | 0.40            | 0.50                 | 30 |
|                            | 45        | 260±22 | 0.52            | 0.24            | 0.46                 | 25 |
|                            | 60        | 260±16 | 0.76            | 0.44            | 0.58                 | 25 |
|                            | 90        | 254±16 | 0.48            | 0.24            | 0.50                 | 25 |
|                            | 120       | 256±20 | 0.37            | 0.13            | 0.36                 | 30 |
| 100% He                    | 10        | 258±19 | 0.53            | 0.07            | 0.13                 | 30 |
|                            | 15        | 251±13 | 0.52            | 0.00            | 0.00                 | 25 |
|                            | 20        | 263±22 | 0.83            | 0.47            | 0.56                 | 30 |
|                            | 25        | 248±13 | 0.68            | 0.08            | 0.12                 | 25 |
|                            | 30        | 257±16 | 0.53            | 0.13            | 0.25                 | 30 |
|                            | 40        | 257±15 | 0.44            | 0.08            | 0.18                 | 25 |
|                            | 45        | 264±20 | 0.73            | 0.23            | 0.32                 | 30 |
|                            | 60        | 240±15 | 0.64            | 0.20            | 0.31                 | 25 |
|                            | 90        | 244±12 | 0.48            | 0.04            | 0.08                 | 25 |
|                            | 120       | 257±15 | 0.48            | 0.04            | 0.08                 | 25 |

Values for wt are means ± SD; N, no. of rats. Dives with N<sub>2</sub>-He-O<sub>2</sub> mixtures (20.9% O<sub>2</sub>) at 175 fsw. Decompression was completed within 10 s. U, undefined value.

than the 95% confidence limit of 10.87 min for bends and 11.78 min for death). The confidence limits on TCHe (lower limits of 1.01 min for bends and 3.40 for death) were too wide to allow such a rejection.

Determination of time-at-depth values to be used in the analysis presents potential problems because of the fast effect of He and O<sub>2</sub>. Small errors in time measurements to be used in the model could produce large differences in estimation, especially of time constants. The analysis presented here treats exposure time as starting when depth is reached and temperature has returned to 28°C. No allowance is made for time during the compression phase or during 5 min of O<sub>2</sub> venting where that procedure is performed. This arbitrary decision was made to simplify the modeling exercise because it was impossible to know exactly the partial pressures of each gas to which the animal was exposed at any given time during compression and venting. However, to estimate errors that could be introduced into the results from the problem with time, a "worst case" data set was constructed. This data set added 3 min to the dive time in all cases and 5 min to the N<sub>2</sub> washout time (movement of the original N<sub>2</sub> out of the animal) in cases where O<sub>2</sub>

was used to vent the chamber at the surface before beginning He-O<sub>2</sub> dives. Obviously the animal was not exposed to the full partial pressures of the gases composing the dive mixture for a full 3-min period during compression, even in the instances where compression took 4–5 min, and not all N<sub>2</sub> is removed from the chamber immediately when O<sub>2</sub> venting is started. Thus these results should produce extreme biases in estimates that can be used as limits to possible errors in the results produced by the analysis. Results from this fitting exercise are presented in Table 4. All parameter estimates, except the time constants, are only slightly different from the original data set (Table 3). For this worst case, the time constants are increased ~1.5 min for He and 2 min for N<sub>2</sub>. Although these changes are substantial, they are not large enough to significantly improve the apparent lag of the predictive curve compared with the data for high N<sub>2</sub> mixtures (see *Predictive Curves*).

#### *Predictive Curves*

Predictive curves derived from the bends and death parameters in Table 3 for the four N<sub>2</sub>-He-O<sub>2</sub> mixtures at 175 fsw used in *series I* are plotted in Figs. 1 and 2; for

TABLE 2. Experimental data from variable time-at-depth dives with rats (series II)

| Gas Composition                                                          | Time, min | Wt, g  | Bends Incidence | Death Incidence | Death-to-Bends Ratio | N  |
|--------------------------------------------------------------------------|-----------|--------|-----------------|-----------------|----------------------|----|
| 141 fswa N <sub>2</sub> + 33 fswa O <sub>2</sub>                         | 10        | 267±14 | 0.00            | 0.00            | U                    | 25 |
|                                                                          | 20        | 262±12 | 0.12            | 0.00            | 0.00                 | 25 |
|                                                                          | 30        | 256±21 | 0.04            | 0.04            | 1.00                 | 25 |
|                                                                          | 45        | 264±12 | 0.48            | 0.16            | 0.33                 | 25 |
|                                                                          | 60        | 259±13 | 0.12            | 0.00            | 0.00                 | 25 |
|                                                                          | 90        | 248±27 | 0.29            | 0.00            | 0.00                 | 24 |
|                                                                          | 120       | 269±16 | 0.52            | 0.16            | 0.31                 | 25 |
| 141 fswa N <sub>2</sub> + 66 fswa O <sub>2</sub>                         | 10        | 256±25 | 0.16            | 0.00            | 0.00                 | 25 |
|                                                                          | 20        | 256±25 | 0.36            | 0.00            | 0.00                 | 25 |
|                                                                          | 30        | 262±20 | 0.24            | 0.04            | 0.17                 | 25 |
|                                                                          | 45        | 245±23 | 0.28            | 0.04            | 0.14                 | 25 |
|                                                                          | 60        | 264±15 | 0.36            | 0.12            | 0.33                 | 25 |
|                                                                          | 90        | 261±17 | 0.52            | 0.36            | 0.69                 | 25 |
|                                                                          | 120       | 259±23 | 0.48            | 0.28            | 0.58                 | 25 |
| 141 fswa N <sub>2</sub> + 99 fswa O <sub>2</sub>                         | 10        | 252±15 | 0.67            | 0.00            | 0.00                 | 24 |
|                                                                          | 20        | 250±25 | 0.56            | 0.08            | 0.50                 | 25 |
|                                                                          | 30        | 249±21 | 0.84            | 0.12            | 0.14                 | 25 |
|                                                                          | 45        | 265±14 | 0.84            | 0.56            | 0.67                 | 25 |
|                                                                          | 60        | 254±25 | 0.88            | 0.56            | 0.64                 | 25 |
|                                                                          | 90        | 257±27 | 0.88            | 0.36            | 0.41                 | 25 |
|                                                                          | 120       | 271±17 | 0.95            | 0.85            | 0.89                 | 20 |
| 141 fsw N <sub>2</sub> + 33 fswa O <sub>2</sub> + 33 fswa N <sub>2</sub> | 10        | 283±17 | 0.07            | 0.00            | 0.00                 | 15 |
|                                                                          | 20        | 266±13 | 0.27            | 0.07            | 0.26                 | 15 |
|                                                                          | 30        | 264±12 | 0.60            | 0.40            | 0.67                 | 15 |
|                                                                          | 45        | 268±20 | 0.73            | 0.53            | 0.73                 | 15 |
|                                                                          | 60        | 275±17 | 0.87            | 0.73            | 0.84                 | 15 |
|                                                                          | 90        | 263±24 | 0.93            | 0.87            | 0.94                 | 15 |
|                                                                          | 120       | 271±17 | 1.00            | 0.93            | 0.93                 | 15 |
| 141 fsw N <sub>2</sub> + 33 fswa O <sub>2</sub> + 66 fswa N <sub>2</sub> | 10        | 241±26 | 0.33            | 0.13            | 0.39                 | 15 |
|                                                                          | 20        | 265±20 | 0.64            | 0.57            | 0.89                 | 14 |
|                                                                          | 30        | 261±29 | 0.87            | 0.80            | 0.92                 | 15 |
|                                                                          | 45        | 275±12 | 1.00            | 1.00            | 1.00                 | 15 |
|                                                                          | 60        | 269±15 | 1.00            | 1.00            | 1.00                 | 15 |
|                                                                          | 90        | 263±15 | 0.93            | 0.87            | 0.94                 | 15 |
|                                                                          | 120       | 272±17 | 1.00            | 1.00            | 1.00                 | 15 |

Values for wt are means ± SD; N, no. of rats. Dives with N<sub>2</sub>-O<sub>2</sub> mixtures (variable % O<sub>2</sub>) at various depths. Decompression was completed within 10 s. U, undefined value.

TABLE 3. Parameters estimated by maximum likelihood technique for Hill equation models of N<sub>2</sub>-He-O<sub>2</sub> dose-response curves

|                        | Bent        | Death       |
|------------------------|-------------|-------------|
| P <sub>50</sub> , fsw  | 154.2±4.2   | 145.3±2.8   |
| n                      | 7.75±0.71   | 12.98±1.14  |
| RPHe                   | 0.957±0.023 | 0.888±0.016 |
| RPN <sub>2</sub>       | 1.0*        | 1.0*        |
| RPO <sub>2</sub>       | 0.877±0.074 | 0.388±0.041 |
| WtF                    | 0.66±0.11   | 0.59±0.09   |
| TCO <sub>2</sub> , min | 0.000001*   | 0.000001*   |
| TCHe, min              | 3.09±1.04   | 4.74±0.67   |
| TCN <sub>2</sub> , min | 13.21±1.17  | 13.40±0.81  |

Values are means ± SE, unless otherwise indicated. Data from Tables 1 and 2 (variable time-at-depth dives). Dose is defined by Eqs. 2-6. Model: probability (bends or death) = dose<sup>n</sup>/(dose<sup>n</sup> + P<sub>50</sub><sup>n</sup>), where Dose is dose of decompression stress, P<sub>50</sub> is depth or pressure that produces 50% incidence, and n is exponent controlling slope of response curve. RPHe, RPN<sub>2</sub>, and RPO<sub>2</sub>, relative potencies for He, N<sub>2</sub>, and O<sub>2</sub>, respectively; WtF, weight factor used to correct dose by weight of animal; TCO<sub>2</sub>, TCHe, and TCN<sub>2</sub>, time constants for effect of O<sub>2</sub>, He, and N<sub>2</sub>, respectively. \* Fixed value.

the five N<sub>2</sub>-O<sub>2</sub> mixtures used in series II, they are plotted in Figs. 3 and 4. Rat weight is held constant at 260 g for all predictions. The 95% confidence limits of these functions are included on the graphs along with the raw incidence data points. In examining how well the predic-

TABLE 4. Parameters estimated by maximum likelihood technique for Hill equation models of N<sub>2</sub>-He-O<sub>2</sub> dose-response curves

|                        | Bent        | Death       |
|------------------------|-------------|-------------|
| P <sub>50</sub> , fws  | 151.4±3.9   | 144.6±2.8   |
| n                      | 8.28±0.74   | 13.19±1.15  |
| RPHe                   | 0.944±0.022 | 0.883±0.017 |
| RPN <sub>2</sub>       | 1.0*        | 1.0*        |
| RPO <sub>2</sub>       | 0.828±0.068 | 0.387±0.040 |
| WtF                    | 0.62±0.10   | 0.57±0.08   |
| TCO <sub>2</sub> , min | 0.000001*   | 0.000001*   |
| TCHe, min              | 4.37±1.12   | 6.24±0.81   |
| TCN <sub>2</sub> , min | 15.33±1.25  | 15.26±0.91  |

Values are means ± SE, unless otherwise indicated. Data from Tables 1 and 2 (variable time-at-depth dives). Same model as in Table 3 (dose defined by Eqs. 2-6) was used but with time corrections for O<sub>2</sub> venting (+5 min) and compression (+3 min). Abbreviations defined in Table 3 footnote. \* Fixed value.

tions agree with the actual data, the confidence limits associated with the data must be used to test for overlap with the confidence limits for the predictive curve. These data confidence limits, obtained from tables of 95% confidence limits for binomial distributions, are also plotted on two graphs in Fig. 1. Examination of the error bars on Fig. 1 emphasizes that uncertainties are large, ranging up to approximately ±20% incidence (based on 14-30 animals/value). Thus large apparent disparities

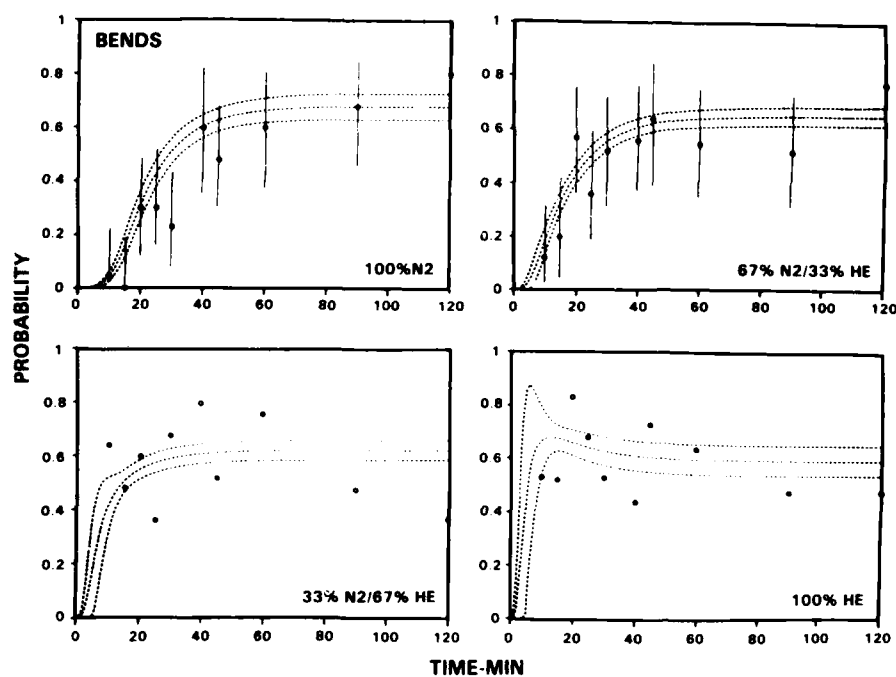


FIG. 1. Probability of decompression sickness (bends) in rats after rapid decompression following variable time-at-depth (time on X-axis) exposures to 175 fswg with 1 of 4 different N<sub>2</sub>-He-O<sub>2</sub> mixtures (20.9% O<sub>2</sub>). Curves are functions ( $\pm 2$  SD, 95% confidence limits) derived from maximum likelihood model given in Table 3, with weight fixed at 260 g. Each symbol is experimentally observed incidence value, based on 25 or 30 animals and on same units as Y-axis. Error bars associated with symbols represent 95% confidence limits based on binomial uncertainty in data.

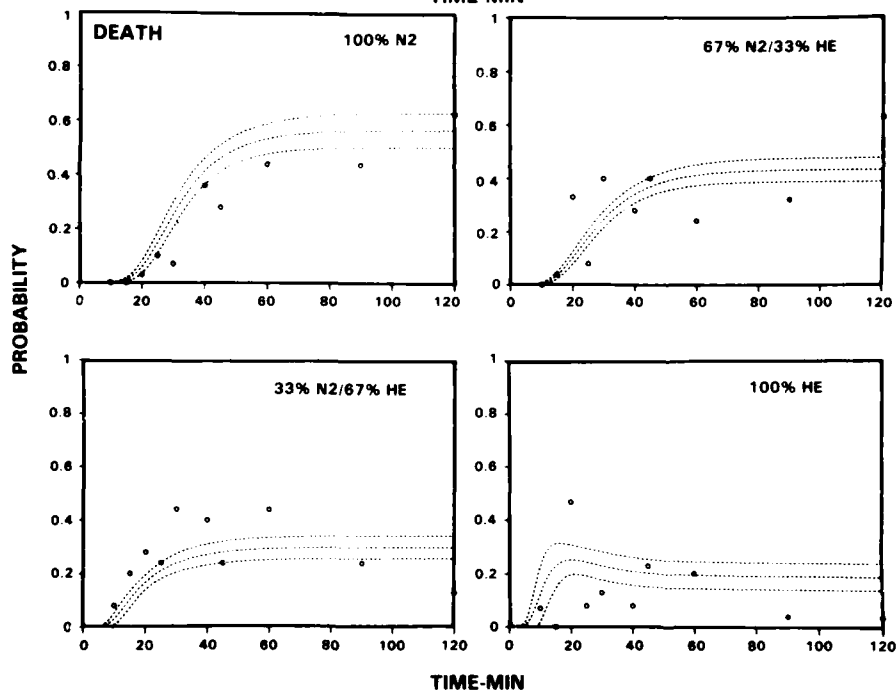


FIG. 2. Probability of decompression sickness (death) in rats after rapid decompression following variable time-at-depth (time on X-axis) exposures to 175 fswg with 1 of 4 different N<sub>2</sub>-He-O<sub>2</sub> mixtures (20.9% O<sub>2</sub>). Curves are functions ( $\pm 2$  SD, 95% confidence limits) derived from maximum likelihood model given in Table 3, with weight fixed at 260 g. Each symbol is experimentally observed incidence value, based on 25 or 30 animals and on same units as Y-axis.

between data points and predictive curves do not necessarily indicate poor fit between model and data. Overall, there appears to be good agreement between the data and the functions (i.e., confidence belts overlap with all but eight data points in Figs. 1-4). This agreement was achieved despite pooling of the data from the two dive series and despite the fact that most rats did not weigh exactly 260 g, which is the value used to define the curve.

As would be expected from differences in TCHe<sub>2</sub> and TCN<sub>2</sub>, the probability vs. time curve for animals breathing He rises to its asymptote much more quickly than

when N<sub>2</sub> was used. The time to reach the actual asymptote is controlled by the gas with the longer time constant, N<sub>2</sub>. Because a washout of the original N<sub>2</sub> in the animal (originally breathing air) is included in the model, all curves will actually approach final equilibrium at the same rate, depending on TCN<sub>2</sub>. Even for He dives, where the curve rises rapidly based on TCHe, there is a later downward movement of the curve with increasing time as a result of the N<sub>2</sub> washout. The final probabilities in Fig. 1 are similar for all four bends curves because of the relatively small difference between the potencies of He

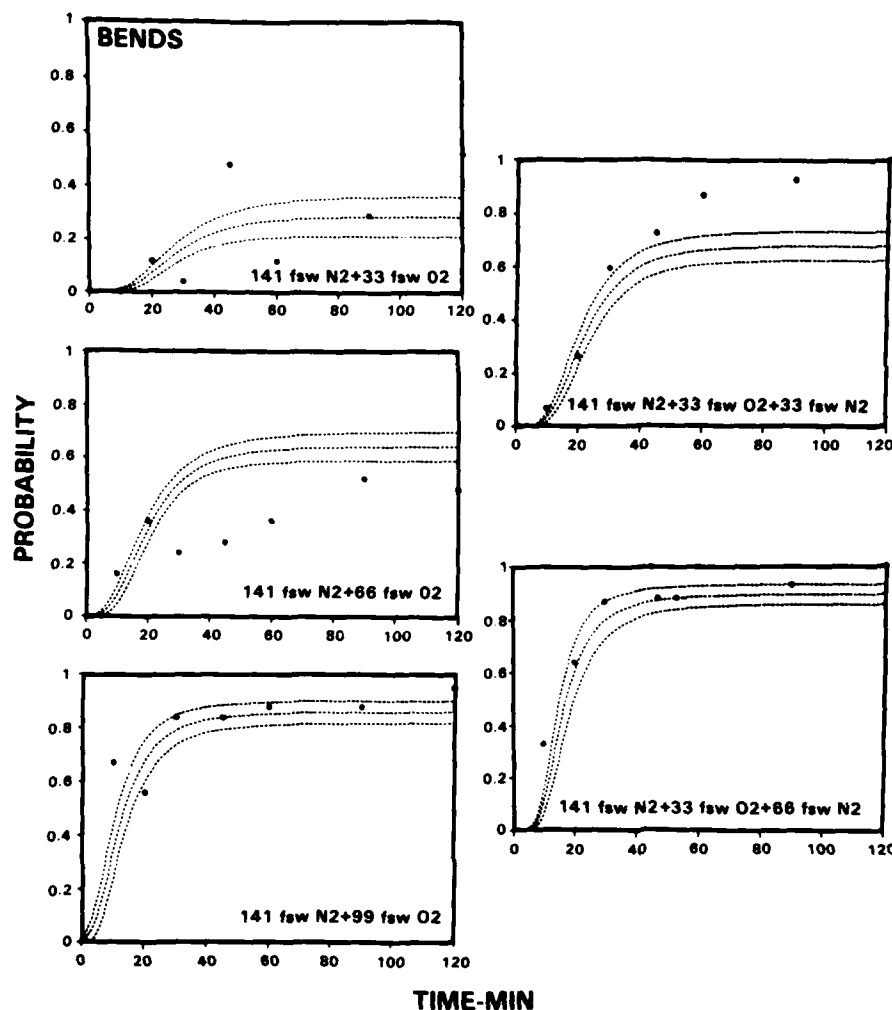


FIG. 3. Probability of decompression sickness (bends) in rats after rapid decompression following variable time-at-depth (time on X-axis) exposures with 1 of 5 different  $N_2$ - $O_2$  mixtures at various depths. All mixtures noted are in absolute units of pressure. Curves are functions ( $\pm 2$  SD, 95% confidence limits) derived from maximum likelihood model given in Table 3, with weight fixed at 260 g. Each symbol is experimentally observed incidence value, based on 14-25 animals and on same units as Y-axis.

and  $N_2$ , whereas the final probabilities are different for death (Fig. 2) because of the large differences in the two potencies. Plots of *series II* experiments (Figs. 3 and 4) do not have the complex kinetics arising from He. The striking feature of these data is the visibly significant contribution of  $O_2$  to increasing the decompression dose. The apparent greater probability of death vs. bends at long dive times for the most potent mixture (i.e., 141 fsw  $N_2$  + 33 fsw  $O_2$  + 66 fsw  $N_2$ ) is not statistically significant.

To demonstrate graphically the effect of  $O_2$  and  $N_2$  on the decompression response, predictive curves with respective 95% confidence limits were constructed for the bends and death responses vs. additional increments (from 0 to 66 fswa of each gas) of  $O_2$  and  $N_2$  added to the starting mixture of 141 fswa  $N_2$  and 33 fswa  $O_2$  (Fig. 5). As before, weight was held constant at 260 g for curve generation, and time was fixed at 120 min. The range of mixtures included those used in *series II* dives. The closeness of the confidence limits for the  $O_2$  and  $N_2$  bends curves reflects the small difference in  $O_2$  and  $N_2$  potencies, unlike the disparity in the relative potencies for death. Again, at the extreme pressures of  $N_2$ , the death curve appears to rise slightly above that for bends.

Experimental incidence values are plotted for comparison.

#### Weight of Animal

Inclusion of a weight correction for the dose resulted in a significant improvement in fit ( $P < 0.01$ ), with heavier animals exhibiting a greater probability for bends or death within the range of depths used. In referring to a weight effect, it is recognized that other biologic processes would be highly correlated with this measure. The alternative sources of the marked interanimal variability include age and time of confinement in our animal-holding facility.

#### Comparison with Saturation Dives

To compare the results of the present study with those of the previous saturation dives (13), a model similar to the present one was used with the saturation dive data. Both methods described the data equally well, and there generally appeared to be good agreement between the two sets of results.

#### DISCUSSION

This investigation has utilized the technique of maximum likelihood to estimate relative potencies for three

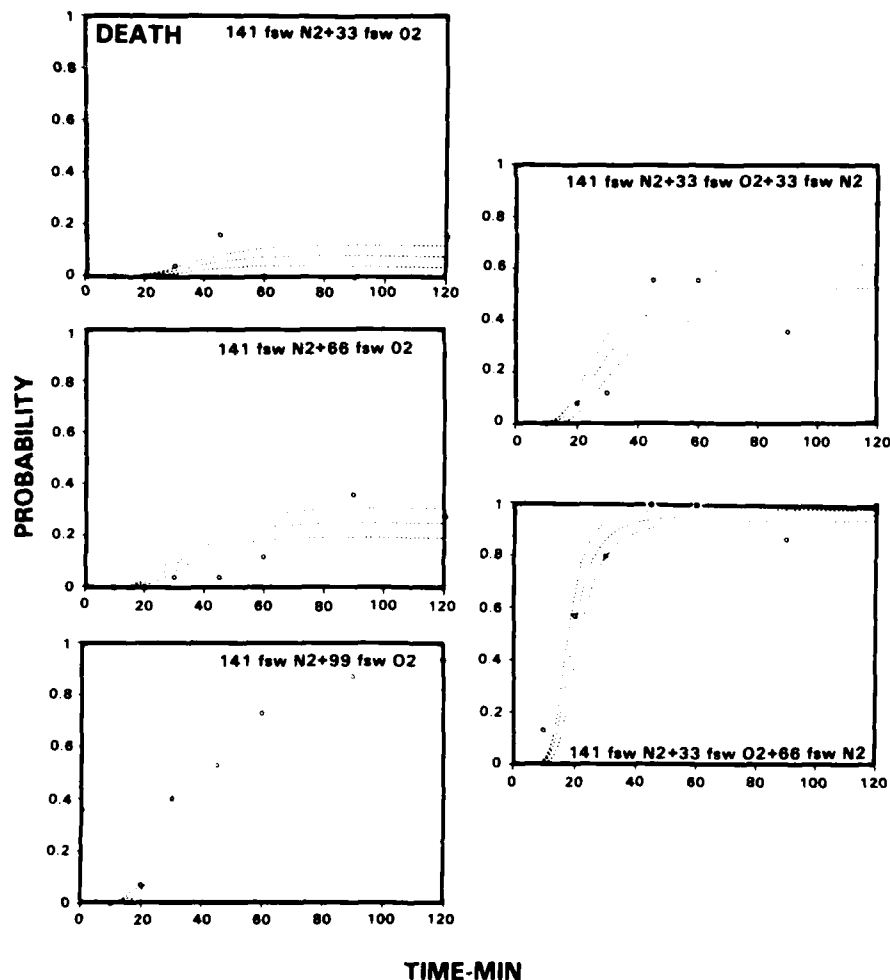


FIG. 4. Probability of decompression sickness (death) in rats after rapid decompression following variable time-at-depth (time on X-axis) exposures with 1 of 5 different  $N_2$ - $O_2$  mixtures at various depths. All mixtures noted are in absolute units of pressure. Curves are functions ( $\pm$ SD, 95% confidence limits) derived from maximum likelihood model given in Table 3, with weight fixed at 260 g. Each symbol is experimentally observed incidence value, based on 14–25 animals and on same units as Y-axis.

gases:  $N_2$ , He, and  $O_2$ . The estimates were found to be of similar magnitude for bends and significantly different for death in the following ascending order of potency:  $O_2 < He < N_2$ . Estimations of time constants of the gases were significantly different, with  $N_2$  requiring a time three to four times that of He. No accurate estimation of  $TCO_2$  could be obtained, although it appears to be considerably shorter than that for  $N_2$  or He.

From these findings, all three gases appear to exert similar effects on decompression outcome for the bends response after dives long enough (i.e., 50 min) to reach saturation. The opposite is true for the death response: because the  $O_2$  potency is lower relative to that of  $N_2$  or He, the decompression load increases with increasing inert gas pressures for a given saturation exposure. For dives  $< 50$  min, both bends and death outcome are influenced by gas uptake rates that affect the partial pressures of individual gases at the time of decompression.

The magnitude of RPHe compares favorably with that found in the saturation dive investigation (Ref. 13; 0.926 and 0.906 for bends and death, respectively), although the present design was not as sensitive to this parameter. Other common parameters in the two investigations were quite similar. A discussion of differences in potency of

inert gases, including He and  $N_2$ , has been presented previously (13).

$O_2$  has long been used to facilitate decompression as well as to treat DCS. Supposedly the benefits of  $O_2$  are derived from two processes: 1) replacement of inert gases in the breathing media to reduce their uptake by the body at depth during a dive and 2) facilitation of gas exchange during decompression by increasing inert gas pressure gradients to hasten removal of these gases. The large negative effect that  $O_2$  apparently had on decompression outcome in rats in these experiments casts doubt on the simplistic view of  $O_2$  as a noninert gas. The extremely rapid decompression used here certainly produces a situation markedly different from normal diving where gas elimination during the decompression phase is facilitated by slow ascent rates that are normally accompanied with periodic decompression "stops." This slower type of decompression may allow much of the  $O_2$  to be metabolized. However, these present experiments agree with those mentioned earlier (6, 15) by suggesting that  $O_2$  has the potential to contribute to the decompression load and question the strict acceptance of the EAD concept. Such a view should at least be qualified to apply only to normal decompression where, with longer de-

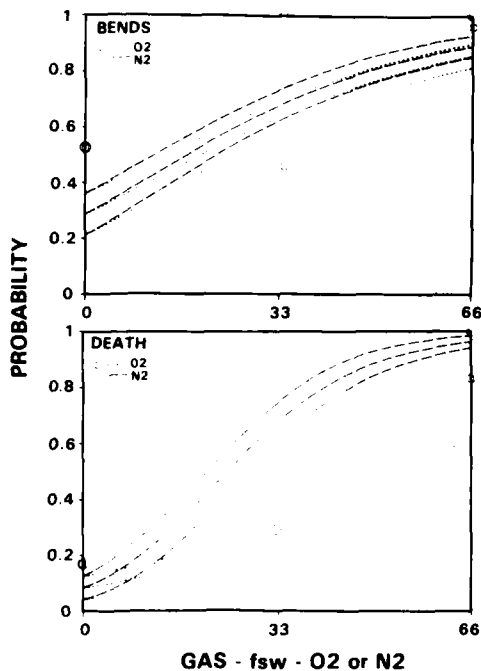


FIG. 5. Predictive curves and respective 95% confidence limits ( $\pm 2$  SD) for effect of increments of  $N_2$  and  $O_2$  on probability of bends and death in rats after 120-min dives based on model in Table 3. Units on X-axis represent amounts of each gas added to starting mixture of 141 fsw  $N_2 \pm 33$  fsw  $O_2$ . Each symbol is experimentally observed incidence value, based on 15–25 animals and on same units as Y-axis. Weight is fixed at 260 g.

compression time, the  $O_2$  effect may well diminish to zero.

Berghage and McCracken (2, 3) concluded that the optimal  $O_2$  level for rat decompression, at very rapid rates, depends on both the ambient hydrostatic pressure and the exposure time with the optimal level declining with increasing exposure time or pressure. This results in a failure of the EAD concept at high  $O_2$  pressures. However, the approach used by Berghage and McCracken (2, 3) of analyzing the data in terms of  $ED_{50}$  determinations with small sample sizes and then fitting models to the  $ED_{50}$  values to predict optimal  $O_2$  responses is extremely questionable. More recently, Yount and Lally (22) have reevaluated the data of Berghage and McCracken (2, 3) using a model incorporating the  $O_2$  window effect (accounting for the inherent unsaturation of venous blood) and concluded that this model agrees with EAD predictions of the decompression advantage offered by relatively low  $O_2$  pressures. However, their analysis also depends on the  $ED_{50}$  values of Berghage and McCracken (2, 3), which must have large variances.

The explanation of the negative impact of  $O_2$  on decompression outcome observed here and by others at high  $O_2$  levels is not known. Berghage and McCracken (3) attributed the lowered decompression tolerance associated with high  $O_2$  pressures to a physiological response such as  $O_2$  toxicity or vasoconstriction rather than to an effect due to gas physics. No symptoms of central nervous system  $O_2$  poisoning, such as convulsions, tremors, or general motor problems, were observed at depth in the current experiments, although animals

were observed only minimally during the dive. Alternatively the possibility exists that  $O_2$  adds to the bubbles, increasing their volume and presumably their damage. At this point, there is no way to rule out any of these possibilities.

No raw decompression outcome data were included in either of the reports by Berghage and McCracken (2, 3), so any evaluation of their findings is dependent on the  $ED_{50}$  values they calculated. These values exhibit comparatively little change over a range of  $PO_2$ 's from 0.2 to 4.2 ATA at a total exposure pressure of 10 ATA for 30-, 60-, and 120-min periods. However, at exposure pressures of 25 and 40 ATA,  $ED_{50}$  values initially increased (indicative of increased decompression tolerance) and then declined considerably as  $O_2$  levels were increased over the same range of 0.2–4.2 ATA. The lowest exposure pressure (10 ATA) conditions were similar to those of the present investigation ( $PO_2$  range: 1.0–3.0 ATA, exposure pressure:  $\sim 5.0$ –7.0 ATA). Thus an unfavorable  $O_2$  effect would not be expected here if the analysis of Berghage and McCracken (2, 3) is used as a guide.

Large differences in rate of effect of He and  $N_2$  have been discussed widely in relation to both human and experimental animal diving (1, 4, 8, 11). However, most previous conclusions are based on statistical methodology and numerous assumptions that make their reliability suspect. Berghage et al. (1), using rats weighing on the average only  $\sim 30$  g more than animals in these dives, estimated that the half time for the  $N_2$  effect on DCS incidence was approximately three times that of He, as was the case in the present study. However, the actual half times reported by Berghage were three times those reported here. Their half times were visually estimated by subjectively drawn curves through points representing mean incidence values, a procedure open to serious error and allowing no statistical interpretation. A similar 1:3 relationship between the half times of He and  $N_2$  has been reported from human diving experiments involving air or He- $O_2$  (20%  $O_2$ ) (5). However, this report appears based on assumptions rather than data fit.

The difficulty of determination of actual numbers of animals needed for experiments such as these has been previously addressed (13). The impact of the actual number of animals would be very dependent on the experimental design, even when using the results of the past study (13) as a guide to probable magnitudes and variabilities of some of the parameters. Thus, in determining sensitivity, not only should the number of animals be considered, but also the distribution of animals over the various variables such as time, gas composition, and depth. The present study relied on the observation that reasonable precision was obtained previously using 25–50 animals for each depth-mixture combination. Nevertheless, Table 3 shows unequal precision in estimated parameters. A different experimental design with more short exposure samples would increase the ability to more precisely estimate  $TCO_2$  and  $TCHe$ ; however, based on earlier work on rats (1), the magnitude of  $TCHe$  was anticipated to be considerably larger and therefore the actual design used was thought to be adequate.

A number of assumptions were made during the modeling process because little is known regarding gas prop-

erties of biologic tissues and physics of bubble development during DCS. The nearly identical results produced by several different methods of defining dose suggest that a fair amount of error in the pressure values that go into the dose calculation can be tolerated by this type of modeling. The similar descriptive ability of the several different models means that mechanisms leading to the different formulations cannot be separated by the present data. Another problem with the present modeling is that the dose calculation is based on static pressure differences. Extrapolation from this model to bubble development then becomes difficult because the pressure differentials are held constant by the model. On the other hand, bubble formation is a dynamic process undoubtedly accompanied by changes in gas pressures. Indeed, the gas composition of intravascular bubbles produced by decompression has been shown to change with time in animals (9), suggesting changes in driving pressures in the body.

Applicability of conclusions from these experiments to human decompression may be limited because of differences in species (rat vs. human), severity of symptoms (death vs. joint pain), and rate of decompression (very rapid vs. staged or slow ascent). However, the protocol used here might model certain human situations such as "blow-up" decompression.

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The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council, Dept. of Health and Human Services, Publ. (NIH) 85-23.

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