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Bubble production in agarose gels subjected to different decompression schedules

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D'Arrigo, J. S., and Y. Mano. 1979. Bubble production in agarose gels subjected to different decompression schedules. Undersea Biomed. Res. 6(1): 93–98.—The relative effectiveness of seven different (military, commercial, and experimental) decompression schedules in reducing bubble formation within aqueous gels has been evaluated quantitatively under rigorously controlled conditions. Specifically, visual counts have been conducted of the bubbles formed in highly purified agarose gels subjected to the different decompression schedules. The order of effectiveness among these schedules in reducing bubble formation in the agarose gel samples was as follows: Model 1 > Royal Naval Physiological Laboratory ≈ French Ministry of Labor >> Yount et al. > Japanese Department of Labor > United States Navy > French Navy. It was concluded that the depth at which slow decompression commences is a major factor, along with the total decompression time, in determining the extent of bubble formation.

decompression schedules
decompression sickness
bubble formation
agarose gels
cavitation
bends

The U.S. Navy Standard Air Decompression Tables (USN) have been, and continue to be, used extensively to guide human decompression procedures throughout much of the world. These tables (United States Navy Department 1974), as well as the French Navy decompression table (FN) and the Japanese Department of Labor standard decompression table (Japan), are all based on the Haldane-ratio principle (Boycott, Damant, and Haldane 1908; Department of Labor, Japan 1976; Beckman 1976; Mano and D'Arrigo 1978). However, other tables have been developed that do not employ the Haldane-ratio principle to guide decompression, and several of these tables appear to be considerably safer (Hempleman 1975; Hills 1975a, 1975b; Beckman 1976).

The Haldane-ratio principle (Boycott et al. 1908) states that it is the ratio of (tissue gas tension)/(ambient pressure) = τ/P_{amb}, and not the difference (or supersaturation pressure) $P_{ss} = \tau - P_{amb}$, that determines how rapidly a diver can surface safely. To compare these points of view, Yount and his co-workers (Yount, Strauss, Beckman, and Moore 1975; Yount and Strauss 1976) have subjected mammalian gelatin samples to simple pressure schedules for which τ/P_{amb} was varied while $P_{ss} = \tau - P_{amb}$ was held constant. Although the difference in the Haldane ratios were large, very little difference was found in the mean number of bubbles formed in the gelatin samples. Accordingly, Yount et al. proposed a more effective decompression procedure that is not based on the Haldane-ratio principle (Yount et al. 1975; Yount and Strauss 1976).

The Royal Naval Physiological Laboratory (RNPL) has also found reason to doubt the Haldane concept of bubble formation and has developed its own decompression schedule (Hempleman 1975). The RNPL table and the decompression table used by the French Ministry of Labor (FL) are based on diffusion theory (Hempleman 1975; Beckman 1976). The most characteristic feature of these tables is the deeper first stop during decompression after diving, with the result that the total decompression time is prolonged to approximately 3 times the period specified by the Haldane-ratio-principle decompression schedules for the same dive (see below). Similarly, a newly developed decompression table (Model 1), used by Nippon Salvage Company of Tokyo in underwater operations during 1975–1976 at Guam (Yano 1978), employs both a deeper first stop and longer total decompression time than do the corresponding USN tables. By using the Model 1 table instead of the USN tables, Nippon Salvage Company was able to reduce the incidence of bends among its divers from over 1.5% to only 0.35% (Mano, Shibayama, Ida, Miyamoto, Matzunaga, Ohgushi, Kashikura, and Maeda 1977; Yano 1978).

In the present experimental study, the relative effectiveness of all the above-mentioned decompression tables in reducing bubble formation within aqueous gels has been evaluated quantitatively under rigorously controlled conditions; specifically, visual counts have been conducted of the bubbles formed in highly purified agarose gels (D'Arrigo 1977; 1978) subjected to the different decompression schedules. The order of effectiveness among the above-mentioned schedules in reducing bubble formation in the agarose gel samples (after a 40-min period of exposure to air at a pressure of 44.5 psig ($\approx$ 100 fsw)) was as follows: Model 1 > RNPL $\approx$ FL >> Yount et al. > Japan > USN > FN.

MATERIALS AND METHODS

Apparatus

The pressure vessel and glass counting chambers and their Plexiglas holder used in these experiments are all similar to those described earlier (D'Arrigo 1978). As viewed through the Plexiglas wall of the pressure vessel, the inside dimensions of each rectangular counting chamber are 15 mm wide and 6 mm along the line of sight.

Each chamber was filled with agarose solution to a depth of 4 mm. After gelation, the agarose samples were exposed to 100-fsw pressures (44.5 psig) for 40 min at 21°C, and then decompressed to atmospheric pressure in accord with one of the seven different decompression schedules tested (listed above). Only bubbles formed in the bottom 3 mm of a given agarose sample were counted, so that the total volume of gel examined in each sample amounted to 0.27 ml.

Solutions

A stock solution containing 1.0 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethane-sulphonic acid, pH_a 7.55) buffer was first prepared. The pH was adjusted to 7.4 by adding small amounts of NaOH. After heating an aliquot of the buffered solution to 80°C, highly purified agarose powder from Bio-Rad, Lot No. 14672 (ash < 0.5%, sulfur content < 0.1%) was added (1% w/w) to the aliquot and dissolved by gently swirling the solution. After 10 min, the agarose solution was transferred to a 50°C bath, from which it was pipetted into the acid-cleaned counting chambers. The unique advantages of using agarose gels to study bubble initiation and growth in aqueous media have already been stressed in preceding studies (D'Arrigo 1977; 1978).

RESULTS

The actual depth profile of each of the seven decompression schedules tested in this study is shown in Fig. 1. For the standard dive chosen of 100-fsw depth and 40-min duration, it can be seen from the figure that there is a wide variation in the decompression profiles specified by the different tables examined. For example, the total decompression times required by the different military and commercial decompression tables ranged between 12.00 min (FN table) and 58.24 min (RNPL table). The shortest total decompression time of only 11.04 min was required by the schedule of Yount et al., which is not a commercial table but rather a pressure schedule derived from that group's model based on their work with mammalian gelatin (Yount et al. 1975; Yount and Strauss 1976).

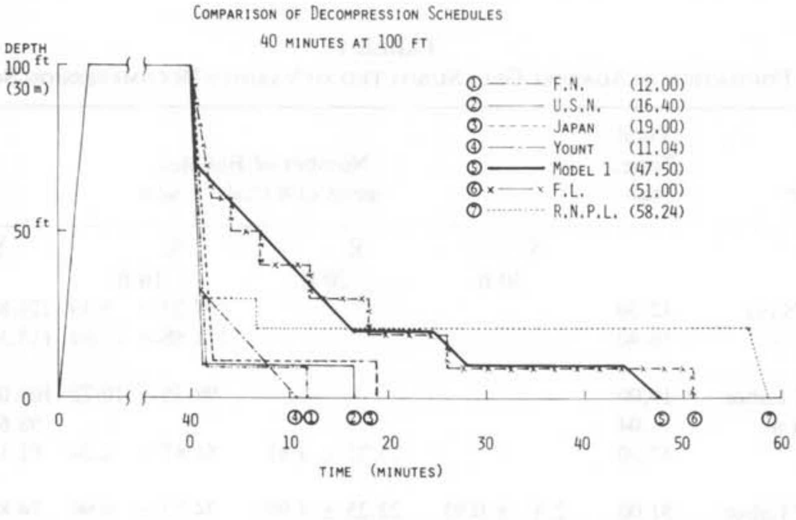

Fig. 1. Comparison of 7 different decompression profiles for standard dive of 100-fsw depth and 40-min duration. Source of each decompression schedule is given at upper right, with total decompression time (min) in parentheses.

In the present study, eight trials using agarose gels were performed for each of the seven decompression schedules, and the total number of bubbles formed per gel sample (0.27 ml) was recorded. Table 1 lists the number of bubbles (mean ± SEM) observed with each decompression profile at various depths, i.e., stops.

DISCUSSION

The separate decompression tables of the French Navy, the U.S. Navy, and the Japanese Department of Labor, which are all based on the Haldane-ratio principle (Boycott et al. 1908; United States Navy Department 1974; Department of Labor, Japan 1976; Beckman 1976), require total decompression times for the test dive which are much shorter than those required by the other military and commercial tables (Table 1). The first stop during decompression with either the FN, USN, or Japan tables occurred at a 10-ft depth, and the mean bubble counts (± SEM) within the 0.27-ml agarose samples just prior to termination of this first (and only) stop were 127.25 ± 9.39, 111.88 ± 17.64, and 98.75 ± 10.72, respectively. Of these three Haldane-ratio-principle tables, the FN table required the shortest total decompression time and the Japan table the longest time, so that the mean bubble number at the 10-ft depth was inversely related to the total decompression time. (In these three cases, the total decompression time essentially represented the sum of the initial period of ascent plus the time spent at the single decompression stop, with both these parameters varying across cases.) The final bubble number (Table 1) was also inversely related to the total decompression time: 129.88 ± 9.59 for the FN table (12.00 min), 115.38 ± 18.28 for the USN table (16.40 min), and 109.00 ± 6.90 for the Japan table (19.00 min).

The shortest total decompression time specified by any of the schedules (11.04 min) was that of the Yount et al. schedule (Yount et al. 1975; Yount and Strauss 1976) for the standard dive. Nonetheless, the final number of bubbles produced by this particular schedule, 98.63 ± 5.91 per agarose sample (0.27 ml), was less than that produced by any of the three tables (FN, USN, Japan) based on the Haldane-ratio principle (Table 1). In particular, the final bubble

TABLE 1

BUBBLE FORMATION IN AGAROSE GELS SUBJECTED TO VARIOUS DECOMPRESSION SCHEDULES

Schedule	Total Time, min	Number of Bubbles, mean of 8 trials ± SEM			
		$\overline{X}$ 30 ft	$\overline{X}$ 20 ft	$\overline{X}$ 10 ft	$\overline{X}$ final
French Navy	12.00			127.25 ± 9.39	129.88 ± 9.59
U.S. Navy	16.40			111.88 ± 17.64	115.38 ± 18.28
Japan, Dept. of Labor	19.00			98.75 ± 10.72	109.00 ± 6.90
Yount et al.	11.04				98.63 ± 5.91
Model 1	47.50		25.75 ± 3.83	54.87 ± 2.24	61.13 ± 1.12
France, Dept. of Labor	51.00	2.5 ± 0.93	22.25 ± 4.90	74.13 ± 6.96	74.87 ± 5.73
RNPL	58.40	12.88 ± 4.76	28.13 ± 7.16		72.38 ± 3.58

Bottom time: 40 min with air at 44.5 psig (≈ 100 fsw); agarose samples - 1% w/w, 0.27 ml in volume.

count produced by the FN schedule was markedly higher ($P < 0.005$), despite the fact that this schedule involved almost the same total decompression time, i.e., 12.00 min. The Yount et al. schedule has no stop during decompression, but instead initiates a slow and constant rate of decompression at about a 40-ft depth (Yount et al. 1975; Yount and Strauss 1976). The starting point for the slow decompression is, therefore, approximately 3.3 times deeper than with the tables (FN, USN, Japan) based on the Haldane-ratio principle (see Fig. 1).

As with the Yount et al. schedule, the Model 1, FL, and RNPL tables are based, at least in part, on diffusion theory (Hempleman 1975; Beckman 1976). Accordingly, these latter three tables also require that slow decompression commence at a greater depth than that specified by the tables based on the Haldane-ratio principle (FN, USN, Japan) (see Fig. 1). The Model 1 schedule initiated slow decompression at the greatest depth (70 ft (Fig. 1)), with the result that the mean number of bubbles produced per 0.27-ml agarose sample (Table 1) was significantly less than the number produced by the FL schedule at the 10-ft stop (54.87 ± 2.24 vs. 74.13 ± 6.96, respectively), and was significantly less than the numbers produced by either the FL schedule or the RNPL schedule at the end of decompression (61.13 ± 1.12 versus 74.87 ± 5.73 and 72.38 ± 3.58, respectively) ($P < 0.05$). This significantly reduced production of bubbles by the Model 1 schedule occurred despite the fact that the total decompression times of the FL and RNPL schedules were longer (47.50 min versus 51.00 min and 58.24 min, respectively). Therefore, as already indicated above by the agarose results obtained from use of the Yount et al. schedule, it is clear that the depth at which slow decompression commences is a major factor, along with total decompression time, in determining the extent of bubble formation. Accordingly, it is the greater magnitude of both of these contributing factors that explains the far fewer bubbles produced in agarose gels by the Model 1, FL, and RNPL tables, as compared with the FN, USN, and Japan tables ($P < 0.01$).

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D'Arrigo, J. S., and Y. Mano. 1979. La production de bulles dans les gels d'agarose exposés à divers schémas de décompression. Undersea Biomed. Res. 6(1): 93–98.—On a accompli, sous des conditions de contrôle rigoureux, l'évaluation quantitative de l'éfficacité relative de sept schémas de décompression (militaires, commerciaux, et expérimentaux) pour réduire la production de bulles. On a compté les bulles visibles formées à l'intérieur de gels d'agarose très purs exposés aux différents schémas de décompression. La liste des schémas testés, rangés en ordre de leur éfficacité, suit:

1) Model 1.
2) Royal Navy Physiological Laboratory, ce qui équivaut à peu pres à celui du
3) Ministère du travail français, qui est par beaucoup supérieur à
4) Yount et al.
5) Japanese Department of Labor
6) United States Navy
7) Marine française

Ces résultats nous permettent de conclure que la profondeur à laquelle la décompression lente est commencée peut être un facteur majeur, avec le temps total de décompression, en déterminant la formation de bulles.

schémas de décompression
maladie de décompression
formation de bulles
gels d'agarose
cavitation
bends (douleurs articulaires de la maladie de décompression)
prédiction de bends

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