Effects of Floating in a Saturated Epsom-Salt Solution on the Aerobic Microbial Flora of the Skin

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ABSTRACT: **The effect of floating in an Epsom-salt solution disinfected with bromine on the microbial flora of three skin sites of 10 subjects is described.**

The 10 subjects floated in a relaxation flotation tank, and samples of the microflora from the subjects' axillas, ankles, and forearms were taken before and after each flotation.

Floating in the Epsom salt-bromine solution produced insignificant reductions in the arithmetic mean counts of the total aerobic flora on the three sample sites. In vitro studies showed that, except for the *Bacillus* endospore, all skin flora isolates were killed within the first few hours of exposure to either the Epsom salt-bromine solution or an aqueous solution of bromine.

The results of this study suggest that microbial transference between subjects in flotation tanks does not occur or is at most insignificant.

Clin Lab Sci 1988;1:358–361

The primary treatment of essential hypertension has been pharmacologic. However, because of side effects encountered in such treatments, a number of nonpharmacologic modalities have been suggested for management of this disease. Of these measures, relaxation training and behavior management seem to be the most effective.[1,2] Another more recent approach involves the use of a flotation tank with restricted environmental stimulation.[3] In this technique, the subject is placed in an enclosed chamber containing a saturated-aqueous solution of magnesium sulfate. Currently, a number of halogen disinfectants are commonly used in commercial applications, of which the most popular are chlorine, iodine, and bromine.

Use of the commercially available flotation tank has increased. Among the commercial claims to the public have been that floating reduces stress, lowers blood pressure, and eliminates the smoking habit.[4–6] Generally, the average therapeutic treatment consists of floating for one hour. At the end of the treatment, the water is circulated rapidly through a course filter before another subject enters the tank.

PURPOSE

This study was conducted to investigate changes in aerobic skin flora and to assess the possibility of cross contamination between patients engaged in flotation studies.

METHODS

Sample

Ten healthy volunteers, three men and seven women, participated in the study. The axilla, forearm (flexor surface), and ankle were selected as sampling sites. The axilla was chosen as an area of high humidity having large microbial populations, while the forearm and ankle were selected as dry areas of low microbial populations.[7]

Materials

The flotation tank, Flotarium (Enrichment Enterprises, Inc., Huntington, NY), was an insulated, fiberglass, egg-shaped structure containing 1,200 pounds of $MgSO_4$ dissolved in 300 gallons of water (48% *w/v*). Water temperature was maintained at 34.2° C (93.5° F). The tank was equipped with a filtration system that removes debris of 10 microns or greater. Water temperature was maintained at 34.2° C (93.5 F). A bromine level of 1.0 ppm was maintained in the 48% salt solution by means of a feeder system containing SPa Brom Feeder Sticks (Hydrotech Chemical Corporation, Marietta, GA).

Procedure

The axilla, forearm, and ankle were sampled by a modification of the detergent-scrub technique of Williamson and Kligman.[8] Each site was sampled in duplicate before and after flotation. Before flotation, a sterile, open-ended, glass cylinder with an internal area of 7.95 cm^2 was placed over the area to be sampled. One mL of 0.1% Triton X-100 in 0.075 *M* phosphate buffer (pH 7.9) was introduced from a sterile pipette into the cylinder. The skin was scrubbed with a sterile blunted glass rod for one minute. Next, the turbid sample was pipetted into a sterile test tube. The procedure was repeated without moving the cylinder, and the two washes were pooled.

The subject then entered the flotation tank and was instructed to keep those areas sampled immersed in the saturated Epsom salt–bromine solution (tank water). After one hour in the flotation tank, the subject was instructed to shower briefly (for 10 seconds) to remove the excess salt. The sampling procedure was then repeated at sites adjacent to the ones previously sampled.

Tank water samples (approximately 1 mL) were transferred to test tubes immediately before and immediately after flo-

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Acknowledgments: To Roderick Borrie for his technical assistance and Enrichment Enterprises, Inc., for their donations and technical support.

tation. The samples were processed by making 10-fold dilutions in 0.05% Triton X-100 in 0.0375 *M* phosphate buffer. Each dilution (0.10-mL volumes) was plated on sheep-blood agar, (Gibco Laboratories, Fairfield, NJ), Trypticase-soy agar (TSA) (Difco Laboratories, Detroit, MI), with 0.5% Tween 80, and Sabouraud-glucose agar (Difco Laboratories). The sheep-blood agar and the TSA-Tween 80 plates were incubated at 35° C for 48 hours and at 30° C for an additional 72 hours. The Sabouraud glucose-agar plates were incubated at 30° C for 10 days. Selected colonies were tested using the Gram stain. Gram-positive organisms were identified by standard methods including macroscopic and microscopic morphology, catalase production, production of spores, oxidative or fermentative metabolism of glucose, and susceptibility to bacitracin. Gram-negative bacteria and yeasts were identified using API 20E and API 20C, (Analytab Products, Inc., Plainview, NY) respectively. Molds were identified by macroscopic and microscopic morphology.

In vitro effects on skin microflora: Tank water—A small amount of the aqueous suspension of the isolate (0.20 mL of a 10^7 CFU/mL) was added to 19.8 mL of tank water. At hourly intervals for eight hours, and then daily for one week; 1 mL was transferred into a sterile capped tube followed promptly by the addition of 1 mL of a sterile 1% aqueous solution of $Na_2S_2O_3$. The purpose of adding $Na_2S_2O_3$ was to neutralize residual bromine and thus prevent continuation of its microbicidal action.[9,10] After mixing, aliquots (0.1 mL) were streaked onto sheep-blood agar and TSA-Tween 80 for bacterial organisms or on to Sabouraud glucose agar for fungi. Sheep-blood agar and TSA-Tween 80 were incubated at 35° C for 48 hours and at 30° C for an additional 72 hours. Sabouraud glucose-agar plates were incubated at 30° C for 10 days.

Aqueous bromine—A concentration of 1 ppm of bromine in 18 mL of sterile distilled water was prepared by adding the bromine salt, SPa Brom, (Hydrotech Chemical Corporation) to distilled water and adjusting its concentration using the Bromine-Duo test (Aquality, Inc., Chatsworth, CA). Then, the aqueous suspension (2 mL of a 10^6 CFU/mL) of the isolate was added; 1 mL of this was immediately transferred to a sterile capped tube followed by the addition of 1 mL of the sterile $Na_2S_2O_3$ solution. This same procedure was followed at hourly intervals for eight hours and then daily for one week. Aliquots (0.10 mL) were then plated onto media and incubated as described above.

48% Salt solution of $MgSO_4$—An aqueous suspension (0.10 mL of a 10^7 CFU/mL) of the isolate was added to 9.9 mL of the salt solution. After mixing, 0.1-mL aliquots were plated onto media and incubated as described above. The process was repeated hourly and daily as above.

Quantitative assessment of tank water microorganisms: A total of 250 mL of tank water was passed through a 0.45-micron filter (Millipore Laboratory Products, Bedord, MA). After the passage of an additional 800 mL of sterile distilled water, the filter was removed and placed onto a sheep-blood agar plate. The plate was incubated at 35° C for 48 hours and at 30° C for an additional 72 hours. This procedure was repeated four times during the study.

Air sampling was performed immediately after the skin flora of each subject was sampled. The procedure consisted of placing uncovered, Sabouraud glucose-agar plates and uncovered, sheep-blood agar plates on the chair where the subject sat during the sampling procedure, on the floor next to the flotation tank, and on the floor in the shower stall for one hour. The sheep-blood agar plates were incubated at 35° C for 48 hours and at 30° C for an additional 72 hours. The Sabouraud glucose-agar plates were incubated at 30° C for 10 days.

Student's *t* test for paired data was used to ascertain whether the difference between the means of the microbial counts obtained before and after each subject floated was significant. The application of this test was limited to the total aerobic-microbial counts and to those groups of microorganisms that were isolated from all 10 subjects.

RESULTS

The total average microbial counts on the three skin sites sampled of the 10 subjects before and after flotation were determined (Table 1). Small decreases in the total counts from all three skin sites after flotation were noted. These reductions were not statistically significant.

The mean numbers of counts per square centimeter of the microorganisms isolated from the three skin sites before and after flotation were compared (Table 2). Coagulase-negative staphylococci was isolated from each of the 10 subjects and from each of the three skin sites sampled. Lower counts were obtained after flotation from the ankle and forearm. However, the axilla showed a relatively small increase in number. The differences in the counts were not statistically significant.

Specimens from the ankle and forearm of five of the subjects and from the axilla of three of the subjects yielded micrococci. Counts from each of the three sites decreased after flotation. Of the three skin sites sampled, only the ankle yielded diphtheroids from all 10 subjects. Cultures of the axilla and forearm from six and three of the subjects, respectively, grew diphtheroids. Axilla and forearm counts were lower after flotation, while counts from the ankle increased. The increased counts observed from the ankle were insignificant.

Specimens from the axilla of two subjects and from the forearms of three subjects yielded increased counts of the *Bacillus* species after flotation. This organism was also isolated from the ankles of four subjects and showed a decrease after flotation. *Moraxella osloensis* was isolated, before flotation, from only one site (the ankle of one subject), while *Rhodotorula rubra* was isolated from two of the three sites sampled. The axillas of four subjects yielded a decreased count of *R. rubra* after flotation, while samples of the

TABLE 1.

EFFECT OF FLOTATION IN A SATURATED EPSOM SALT-BROMINE SOLUTION[a] ON THE ARITHMETIC MEANS OF TOTAL AEROBIC MICROBIAL COUNTS PER SQUARE CENTIMETER ON THREE SKIN SITES

	Total counts/cm²	
Site	**Before flotation**	**After flotation**
Axilla	1.3×10^5	1.0×10^5
Ankle	1.4×10^2	1.0×10^2
Forearm	1.0×10^2	9.4×10^1

[a] 48% aqueous $MgSO_4$ containing 1 ppm bromine.

TABLE 2.

EFFECT ON THE AEROBIC MICROBIAL FLORA OF FLOATING IN A SATURATED EPSOM SALTS-BROMINE SOLUTION[a]

	Average counts/cm² (no. isolates)						
	Coagulase negative *Staphylococci*	*Micrococcus* species	Total diphtheroids[b]	*Bacillus* species	*Moraxella osloensis*	*Rhodotorula rubra*	*Penicillium* species
Axilla							
Before flotation	5.8×10^4 (10)[c]	2.1×10^2 (3)	6.8×10^4 (6)	1.1×10^1 (2)	0	7.6×10^4 (4)	0 (1)
After flotation	7.3×10^4	8.4	2.9×10^4	3.2×10^2	0	3.8×10^1	5.0×10^2
Ankle							
Before flotation	1.2×10^2 (10)	1.8×10^1 (5)	2.5 (10)	2.2 (4)	1.26 (1)	0 (2)	0 (2)
After flotation	7.1×10^1	7.2	1.6×10^1	0.02	0	7.2	1.26
Forearm							
Before flotation	5.8×10^1 (10)	1.7×10^1 (5)	2.7×10^1 (3)	1.26 (3)	0	0	0 (2)
After flotation	4.4×10^1	6.6	6.1	8.8	0	0	2.8×10^1

[a] 48% aqueous $MgSO_4$ containing 1 ppm bromine.

[b] Lipophilic and nonlipophilic diphtheroids.

[c] Numbers in parenthesis indicate number of subjects from which the organism was isolated.

ankles of two subjects grew this yeast only after flotation. Another genus, *Penicillium*, was isolated only after flotation from the ankles and forearms of two and from the axilla of one subject.

The microbicidal activity of tank water and its constituents on isolated skin flora were also determined (Table 3). The microbicidal activity of aqueous bromine (1 ppm) on the isolated skin microflora paralleled the activity observed with the tank water. Incubation in tank water killed the *Penicillium* spore within three hours, and all vegetative bacterial cells were killed immediately. However, the *Bacillus* endospore survived in the tank water for the length of the study.

The *Bacillus* endospore survived exposure to the high concentration of aqueous $MgSO_4$ for the seven-day study period. Although other skin-flora isolates survived longer in the salt solution than in the tank water and bromine solution, all were killed within 48 hours. In addition, aliquots (0.10 mL) of the tank water, removed before and after each subject floated, did not yield any viable microorganisms. The average microbial count of five separate passages of 250 mL of tank water through a 0.45 micron filter was 0.068 CFU/mL. The predominant isolate was coagulase-negative staphylococci. Smaller quantities of micrococci, *M. osloensis*, and α-hemolytic streptococci were also isolated. The predominant airborne microorganism isolated in the flotation tank room was *Rhodotorula rubra*. The *Penicillium* species, *Aureobasidium pullulans*, and the *Cladosporium* and *Micrococcus* species were also isolated.

DISCUSSION

This is the first study of the effect on the cutaneous microflora of floating in a relaxation tank containing a saturated aqueous solution of magnesium sulfate disinfected with bromine. Although bromine[10,11] and concentrated-salt solutions[12,13] have marked antimicrobial activities, the total number of aerobic microflora did not decrease significantly after

TABLE 3.

EFFECT OF TANK WATER, SALT SOLUTION, AND BROMINE SOLUTION ON SKIN MICROBIAL ISOLATES

Organism (10^5 CFU/mL)	Microbicidal end point (hours)		
	Tank water (48% aqueous $MgSO_4$ containing 1 ppm bromine)	Salt solution (48% aqueous $MgSO_4$)	Bromine solution (Distilled H_2O with a concentration of 1 ppm bromine)
Coagulase-negative staphylococci	0[a]	5	0
Micrococcus species	0	24	0
Total diphtheroids[b]	0	1	0
Moraxella osloensis	0	48	0
Bacillus species (vegetative)	0	1	0
Bacillus species (endospore)	N.E.[c]	N.E.	N.E.
Rhodotorula rubra	0	6	0
Penicillium species	3	24	2

[a] Immediate kill.

[b] Lipophilic and nonlipophilic diphtheroids.

[c] No effect on viability at seven days.

flotation in these solutions. Except for the *Bacillus* endospore, the sensitivity of the isolated-cutaneous microflora to the tank water and to its individual components suggested that the exposed-skin microflora would be rapidly killed during flotation. The absence of significant change in the total number of cutaneous microflora may have resulted from either a neutralization of the microbicidal activity of the tank-water disinfectant or from insufficient contact of the microbes with the tank water. The microbicidal activity of the tank water was demonstrated by the culture negative results obtained from the water before and after each subject floated and by the water's low microbial load. Therefore, it is reasonable to assume that the principal mechanism preventing a significant drop in the cutaneous microflora after flotation was a shielding of the flora from the tank water. Studies have shown that significant numbers of resident skin microflora are protected from mechanical and chemical assault by lipids and cutaneous debris and by the natural architecture of the skin.[14–16]

Aerobic-skin flora have been shown to consist of members of the genera *Staphylococcus, Micrococcus, Corynebacterium* (diphtheroids), and *Pityrosporum*.[7,17,18] Except for the staphylococci isolated from the axilla and the diphtheroids isolated from the ankle, the resident flora were found in lower numbers at all sites after flotation. The increases observed in the resident flora following flotation were not significant. The reduction in counts of staphylococci from the ankle and the forearm were also insignificant. The remaining members of the resident flora showed relatively small decreases after flotation.

The other groups of microorganisms are usually considered nonresident or transient flora.[14] With the exception of *Moraxella,* commonly isolated from human and lower animal mucous membranes,[19] these organisms have been categorized as environmental airborne contaminants.[14,20] In fact, two organisms, *R. rubra* and the *Penicillium* species, were found to be present in the test environment. Therefore, the significance of effect of flotation on their numbers is difficult to evaluate. However, the decrease in the counts after flotation observed with the nonresident flora were relatively small and of a similar magnitude as that seen with the resident flora.

The increase in the nonresident flora after floating were also relatively small. Among the filamentous fungi, this increase was reflected by their appearance after floating. This may have resulted from the spores in the environment being set in motion as a consequence of the physical activities that occurred during and after the experimental procedures.

CONCLUSION

Several conclusions may be drawn from this study. First, the aerobic-skin flora is not significantly altered after immersion in the saturated Epsom salt–bromine flotation fluid. Second, the microbicidal activities of the tank water and its components on the majority of the skin isolates, as well as the low microbial load of the tank water, suggest that microbial transference between subjects does not occur or is at most minimal.

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