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Effect of Diving and Diving Hoods on the Bacterial Flora of the External Ear Canal and Skin

ITZHAK BROOK,[1]* JAMES C. COOLBAUGH,[1] AND ROBERT G. WILLISCROFT[2]

Naval Medical Research Institute, Bethesda, Maryland 20814,[1] and National Oceanic and Atmospheric Administration, Atlantic Marine Center, Norfolk, Virginia 23510[2]

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The bacterial flora of the external ear canals and posterior auricular skin surfaces was investigated in a group of 26 divers after 25 dry-suit dives in harbor water and 20 dry-suit dives in clear test tank water. A control group of 16 divers wore rubber hoods 19 times for a similar period (25 to 30 min) but did not dive. The protective effect of 2% acetic acid was tested by instilling it in the left ear of 14 divers and 8 nondivers. *Staphylococcus epidermidis, Propionibacterium acnes*, alpha-hemolytic streptococci, and enteric gram-negative rods were the predominant isolates from skin and ear samples. After the divers dove or after they wore hoods without going in the water, there was a substantial increase in the number of these organisms on the skin (46.9%) or in the external ears (43.8%) of the divers. However, an increase in the bacterial counts in the external ear canals occurred in only 13.6% of the individuals treated prophylactically with acetic acid drops. Although no gram-negative rods were recovered from the skin or external ear canals of divers in clear tank water, 23 strains were isolated after the dives in harbor water. Identical gram-negative isolates also were recovered from the harbor water. Gram-negative organisms also were recovered from three newly acquired skin lacerations, where they persisted for at least 24 h. Our data show the acquisition of gram-negative rods when dives were made in polluted water. The data also demonstrate the increase in bacterial counts that occurs when rubber diving hoods are worn (in or out of water) and that this increase can be controlled by pretreatment of ears with acetic acid.

Previous studies have demonstrated the association of external otitis with an increase of gram-negative rods in the external auditory canal (2, 8), especially during dives (11). Diving in polluted water exposes the external ear to contamination by the organisms present in the water. Furthermore, the elevated temperature common inside a rubber diving hood induces increased perspiration and a subsequent increase in humidity which could predispose the skin inside and outside the ear canal to infection. The trauma of removal of cerumen by divers may also induce external ear infection (9).

To avoid external ear infection, divers frequently instill acetic acid ear drops into the ear canals; however, the value of this procedure has not been studied with quantitative cultures of the external auditory canals (11).

In this study we investigated the changes in the bacterial flora of the external ear canals and the skin adjacent to the ear lobes of divers who performed work in polluted and unpolluted waters. We also investigated the effect on the bacterial flora of divers who wore rubber hoods but did not dive and tested the efficacy of acetic acid ear drops in modifying changes in flora.

MATERIALS AND METHODS

Diver population. The participating divers were officers in the commissioned corps of the National Oceanographic and Atmospheric Administration. All were professional divers who dove routinely but who had not dove for at least 4 weeks before these experiments. All were males between 24 and 38 years old and were in good physical condition. None had received any antimicrobial therapy for at least 6 weeks. Otolaryngological examination of the divers before the experiment revealed no evidence of external or internal ear infections. Variable-volume dry suits with tight hoods were used.

The population studied was divided into two groups: divers and controls. There were 26 divers who participated in 45 dives of 25- to 30-min duration (25 dives were performed in harbor water [Norfolk, Va.] and 20 were performed in clear tank water [White Oak, Md.]). The control group included 16 divers who put on hoods on 19 occasions for 25 to 30 min while engaged in diving support work out of the water.

Locations of experiments. The experiments were conducted in three parts at two sites. Experiments 1

and 3 were performed for 2 days each time during January and May 1981 at the Atlantic Marine Center on the Elizabeth River, Norfolk, Va. Maximum diving depth was about 24 ft (731.52 cm). Experiment 2 was conducted for 5 days during February 1981 in a 104-ft (3,169.92-cm)-deep test tank at the Naval Surface Weapons Center, White Oak, Md.

Microbiological assays. Water samples were collected from all diving sites and were cultured for aerobic and anaerobic organisms. Samples for bacterial culture were collected from the divers immediately before they entered the water. Specimens were obtained from the right external auditory canals in all instances and from the left ears of 14 divers and 6 controls. The specimens were obtained with a sterile cotton swab moistened with sterile saline. The external auditory canals were swabbed gently by rolling the swab 360 degrees. The flora of the skin was sampled by swabbing an area of approximately 1 cm^2 behind the right ear lobes with a saline-wetted sterile cotton swab. After the specimens were collected, the swabs were streaked directly onto sheep blood agar plates (enriched with vitamin K_1 and hemin; 5) for isolation of aerobic bacteria (experiments 1 and 2) and onto brain heart infusion agar for isolation of anaerobes (experiment 1).

The swabs were introduced into Cary-Blair transport broth (1) for 24 to 48 h for transportation only in experiment 3. Quantitative bacterial counts were done by plating serial dilutions of the Cary-Blair broth. Although data on the reliability of Cary-Blair medium for quantitative cultures of all pathogens are not available, this medium was chosen because of its excellent performance in field trials (3). Semiquantitative bacterial counts were done by visually grading the growth on streaked plates on a scale of 1+ (light growth) to 4+ (heavy growth).

Aerobic cultures were done in all three experiments, but anaerobic cultures were done only in experiment 1. Aerobic isolates were identified by conventional methods (7). Processing for identification and quantification of anaerobic bacteria was done as follows. All media and dilution blanks were prereduced by incubation at 25°C in an anaerobic glove box (Germfree Laboratories, Inc., Miami, Fla.), transported to the sampling site in anaerobic GasPak jars (BBL Microbiology Systems, Cockeysville, Md.), inoculated, placed in GasPak jars to maintain the reduced state, and returned to an anaerobic glove box. Anaerobiosis in the glove box was maintained with a gas mixture of 85% N_2, 10% CO_2, and 5% H_2 constantly circulated through HEPA filters over palladium catalysts equipped with an FCS (filtration catalyst system; Germfree Laboratories). Resazurin solutions were used as indicators of anaerobiosis. Obligately and facultatively anaerobic bacteria were differentiated by replicating these plates twice, incubating one set at 35°C in an atmosphere containing 10% CO_2, and incubating the other set anaerobically at 35°C for 48 h. Obligate anaerobes were identified on the basis of Gram stain morphology, gas-liquid chromatography of metabolic by-products, and the Minitek (BBL) anaerobic identification scheme (4).

Acetic acid application. Four drops of 2% acetic acid solution were instilled in the left ears of 14 of the divers and 8 of the control group immediately after specimen collection (in experiments 2 and 3). The recipients were instructed to tilt their heads with the left ears up for 5 min before diving or donning their hoods.

RESULTS

Bacterial growth was noted in all of the ear canals and skin specimens. Semiquantitative examination generally showed 2+ to 4+ growth. Those specimens quantified by tube dilution generally had counts of between 10^3 to 10^6 organisms per specimen.

The organisms uniformly found to colonize the external ear canals and the adjacent skin surfaces were *Staphylococcus epidermidis* (in 67% of each of these sites), *Propionibacterium acnes* (in 34% of skin and 17% of ear canals), and alpha-hemolytic streptococci (in 25% of skin and 18% of ear canals). The recording of changes in flora in the experiments was based on changes that occurred in the rates of recovery of only these organisms. A change in flora was judged to occur when a difference of at least 2+ or 1 log was noted between consecutive cultures of an individual site.

Gram-negative enteric organisms were recovered from external ear canals and skin samples only in the experiments which were conducted in harbor water (Table 1). No gram-negative organisms were isolated from divers in clean water.

Of the 23 (78%) gram-negative enteric organisms that were recovered from skin and external ears, 18 were recovered in mixed culture with other enteric or gram-positive aerobic or anaerobic organisms.

Before submergence in Norfolk Harbor (experiment 2), two of the divers acquired superficial skin lacerations (one of them acquired two lacerations). These divers exposed their hands to the water during submergence. Specimens from the lacerated areas were obtained before the dive, after the dive, and 24 h later. The bacteria found in these wounds are listed in Table 2. The three wounds became colonized with gram-negative enteric organisms which were also recovered from the water.

The changes that occurred in the total number of bacteria after the divers dove or wore hoods but were not submerged are summarized in Table 3. There was a substantial increase in the number of divers whose skin or external ear flora increased (48.9% and 46.7%, respectively). A similar increase in skin and ear flora also was noted in those who wore hoods but did not enter the water (42.1% and 36.8%, respectively). Only 2 of the 14 divers (14.3%) whose left ears were pretreated with acetic acid showed an increase in bacterial flora in the treated ears. Although the number of gram-positive bacteria decreased in treated ears, newly isolated strains of gram-negative organisms were recovered from two

TABLE 1. Number of strains of gram-negative rods isolated from external ear canals and skin of 17 divers performing 25 dives of 25 min each in Norfolk Harbor and a group of 4 nondivers who wore hoods eight times for 25 min

Organisms	No. of strains of gram-negative organisms isolated before dive (25)[a]		No. of strains of gram-negative organisms present after:			
			Dive (25)		Hood was worn (8)	
	From skin	From ears	From skin	From ears	From skin	From ears
Pseudomonas aeruginosa			2	3		
Pseudomonas paucimobilis			1			
Pseudomonas putrefaciens				1		
Pseudomonas fluorescens		1	2[b]	1		
Pseudomonas maltophilia			1	1		1
Pseudomonas sp.		1	1[b]			
Chromobacter sp.				1		
Pasteurella sp.			1[b]			
Citrobacter sp.			1			
Alcaligenes sp.			1[b]	2	1	
Serratia sp.	1					
Enterobacter agglomerans	1					
Enterobacter aerogenes				1		
Klebsiella pneumoniae			1	1[c]		
Escherichia coli			1[b]			

[a] Numbers in parentheses indicate number of sites tested.
[b] One strain isolated from skin laceration exposed to water.
[c] Diver developed external otitis media 5 days after dive.

divers. However, these two newly isolated gram-negative rods were not recovered on subsequent cultures done 3 and 24 h after the dive.

A comparison of the acetic acid-treated and nontreated groups (divers and nondivers) revealed that 43.8% of the untreated individuals had an increase in bacterial counts in the ears after the experiment, whereas only 13.6% of the treated individuals showed a similar increase. This difference was significant at $P < 0.02$ by chi-square analysis. On the other hand, there was no significant difference ($P > 0.05$) between increases in bacterial flora in divers (43.8%) compared with nondivers wearing hoods (39.5%).

Although total increases in bacterial counts appeared to be similar in hooded nondiving and diving individuals, there were differences in the number of specific gram-negative isolates from each group. As shown in Table 1, only four gram-negative isolates were recovered from the skin or external ears of divers before submergence. However, after submergence, the number of isolates increased to 12 from skin and 11 from ear canals (total, 23). Identical gram-negative isolates were recovered from the water samples at the diving site. In contrast, only two gram-negative isolates were recovered from those who wore hoods but did not dive in the harbor water.

TABLE 2. Bacterial isolates from skin lacerations of two divers[a]

Diver	Bacterial isolates found:		
	Before dive	After 30-min submergence	After 24 h
1	NG	*Citrobacter* sp. (10^5)	ND
2			
Wound 1	*Staphylococcus epidermidis* (10^3)[b]	*Staphylococcus epidermidis* (3×10^5)	*Staphylococcus epidermidis* (10^5)
		Pseudomonas sp. (5×10^5)	*Escherichia coli* (4×10^4)
		Pseudomonas fluorescens (5×10^5)	*Alcaligenes* sp. (2×10^5)
Wound 2	ND	*Pseudomonas paucimobilis* (1.3×10^4)	*Pseudomonas paucimobilis*
			Pseudomonas sp. (3×10^6)

[a] Abbreviations: NG, no growth; ND, not done.
[b] Numbers in parentheses indicate colony-forming units of isolate per specimen.

TABLE 3. Changes in bacterial flora in skin and external ear canals and the effect of application of acetic acid after divers dove or wore diving hoods[a]

Source of bacteria and experimental conditions	No. of divers experiencing:		
	Increase in the no. of bacteria	Decrease in the no. of bacteria	No change in the no. of bacteria
Skin			
After dive	22 (48.9%)	7 (15.5%)	16 (35.6%)
No dive, but hood was worn	8 (42.1%)	3 (15.8%)	8 (42.1%)
Right ear canals (untreated)			
After dive	21 (46.7%)	2 (4.5%)	22 (48.8%)
No dive, but hood was worn	7 (36.8%)	1 (5.4%)	11 (57.8%)
Left ear canals (treated)			
After dive	2 (14.3%)	9 (64.3%)	3 (21.4%)
No dive, but hood was worn	1 (12.5%)	3 (37.5%)	4 (50%)

[a] Twenty-six divers performed 45 dives, and 16 nondivers wore diving hoods 19 times. A change in counts was defined as an increase or decrease greater than 1 $\log_{10}$ colony-forming unit or semiquantitatively, 2+ or more during the 25- to 30-min experiment time. Numbers in parentheses indicate percentage of total.

DISCUSSION

Our study investigated the effect on the bacterial flora of the external ear canals and adjacent skin of wearing diving hoods in and out of the water. We demonstrated that in both sites an increase in bacterial counts occurs after a short time (25 to 30 min) whether the hoods are worn in or out of clean or relatively polluted water. This increase can be prevented by pretreatment with acetic acid.

The short time needed for this phenomenon to occur makes it unlikely that the increase in bacterial numbers can be explained by multiplication of the organisms. A more logical explanation is that the increase is induced by the outpouring of the resident organisms from the deeper skin layers and the sweat and sebaceous glands. This outflow of organisms can be facilitated by the heat and increase in humidity that occurs during the physical stress of diving and the tight occlusion produced by the hood.

A relationship between the increase in the number of normal flora and skin or external ear infections has not yet been established. However, an increase in bacterial numbers, accompanied by skin macerations or lacerations, could promote an infection. It is interesting that an increase in bacterial counts, identical to that seen in divers, also occurred in nondiving personnel who wore hoods. Hoods and ear protection devices are used in professions other than diving, but whether this practice is associated with a possible risk that can predispose a person to external otitis has yet to be determined.

Additionally, we found that even though the divers were wearing dry suits to protect them from water contamination, they acquired gram-negative organisms after diving in harbor water. These organisms, which were also recovered from the water at the diving sites, are capable of causing external otitis media (11), skin infections (6), and diarrheal diseases (10). One aspect of their potential pathogenicity was demonstrated in our study by their ability to colonize the three exposed skin wounds of two of the divers. Although the hoods of the diving suits fit tightly and supposedly keep the ears and skin of the divers dry, minute amounts of polluted water could reach the skin and external ear canals to initiate the process of colonization and subsequent infection.

Acetic acid has been used for the treatment and prevention of external otitis (9). The ability of acetic acid to reduce or prevent an increase in bacterial flora was demonstrated in our study. Although on two occasions gram-negative bacteria were recovered from the pretreated ears immediately after the divers emerged from the water, the organisms subsequently failed to colonize the external auditory canals. Our findings thus suggest the potential efficacy of acetic acid in preventing changes in bacterial counts and aborting acquisition of potential pathogens.

Our study demonstrated the risks involved in wearing head hoods in or out of water. It also demonstrated the hazards involved in submergence in polluted water and a possible failure of currently used diving suits to prevent acquisition of gram-negative organisms. Further studies are needed to investigate the phenomenon of the increase in bacterial flora during diving and to control the acquisition of pathogenic gram-negative organisms. Such studies will decrease the risk of infection to divers in clean or polluted waters.

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