LETTERS TO THE EDITOR

To the Editor:

Microbiologic hazards for inhabitants of deep diving hyperbaric complexes

Crews stay in deep-diving hyperbaric complexes (DDHC) for several days to months. In such conditions, development of infectious diseases is one of the main sources of trouble, often requiring medical decompression. The most common infectious diseases for DDHC crews are skin infections, acute nasopharyngitis, tracheitis, and otitis media and externa (1–3). During saturation diving work, external otitis is considered to be the most frequent pathology (21%) (4, 5).

This problem persists even when general aseptic and antiseptic measures are scrupulously observed by DDHC crew members and when the DDHC internal surfaces, interior, and equipment are disinfected prophylactically and during use.

We approach the problem by using the methodology and experience accumulated by hygienists and microbiologists during long-term investigations of space habitation. We presume that common factors are acting in space and in underwater habitations. The general purpose of our work was to determine the effect of the DDHC environment on human microflora, and to determine the most evident sources of infections and how they are spread in DDHCs. Our investigations were carried out during several simulation studies and during deep diving from sailing ships equipped with DDHCs.

Microflora of divers’ noses, ears, and throat cavities, skin and meata acoustica externa, and the interior and equipment surfaces of the DDHC were studied several times during one deep dive: at Day 0, during Day 1 at pressure, and then every 7 days up to the end of decompression. The samples were gathered by special cotton swabs; the microbial content of the samples was studied by generally accepted methods.

Microbiological samples were decompressed, then dissolved 10-, 100-, and 1000-fold and each solution was inoculated on the following solid media: blood agar, bromthymol blue agar, citrate agar, and Sabouraud media. After incubation, the grown colonies were counted and pure cultures were isolated and identified in accordance with Houlé (6). Isolation and biotyping of Pseudomonas aeruginosa was in accordance with Moroz (7).

The data suggest that the microbiological situation in habitated baro chambers is rather specific. In all the investigations dealing with long-term dry-diving simulation studies and in deep diving, Gram-negative rods (GNRs) spread intensively. At Day 0, GNRs are rarely detected, but in high-pressure conditions GNRs were detected on all wet surfaces of the DDHC equipment and interior, in the air of the chamber, on divers’ equipment, and in the mucus of divers’ noses, throats, and mouth cavities and on the surface of the internal auditory meatus. The GNRs were mainly represented by the following species and genera: Achromobacter, Alcaligenes, Acinetobacter, Flavobacter, Escherichia coli, Enterobacter liquefaciens, E. aerogenes, E. cloaceae, Hafnia, Klebsiella ozenae, P. capacita, P. aeruginosa. The latter, being an
obligate pathogen, usually predominated in all the gathered samples. Tables 1–3 and Fig. 1 illustrate the process of *P. aeruginosa* ecological spread in the DDHC during deep diving. *P. aeruginosa* colonization of mucous envelopes and skin usually is followed by manifestations of infection, for in the majority of observed deep dives, several divers from different groups suffered from otitis externa, with virulent *P. aeruginosa* strains isolated from their external ears.

Probably GNRs are able to adapt effectively to high pressure conditions; Gram-positive rods and cocci do not possess this characteristic. In several laboratory experiments it was shown that during the first 48 h of incubation in high pressure, the growth of *Staphylococcus aureus* and *P. aeruginosa* PAO 103 was suppressed dramatically. Seventy-two hours later, the number of *P. aureus* colonies and colonies that reached “start” characteristics differed greatly from the control ones. At the same time, within a *P. aeruginosa* strain, dissociation took place, and as a result, half of the clones formed large, wide colonies for which high pressure is no longer a limiting factor (Table 4). Besides this remarkable reproductive capacity, the pathogenicity and defense enzymes (lecithinase, hemolysin, and others) are twice as active as control and barosusceptible clones. At the same time, the representatives of coccal clones produced pathogenicity and defense enzymes less actively in comparison with control clones. Tremendous changes were shown, for instance, in lecithinase

### TABLE 1
**NUMER OF CREW MEMBERS CONTAMINATED WITH P. AERUGINOSA**

<table>
<thead>
<tr>
<th>Exposed Surface</th>
<th>Zero Day</th>
<th>At Pressure</th>
<th>P Values of Differences</th>
<th>CFUs per Swab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nose Cavity</td>
<td>0 out of 10</td>
<td>4 out of 10</td>
<td>&gt;0.05</td>
<td>2 × 10^2–1 × 10^4</td>
</tr>
<tr>
<td>Throat &amp; mouth cavity</td>
<td>0 out of 16</td>
<td>12 out of 16</td>
<td>&lt;0.010</td>
<td>4 × 10^3–4 × 10^3</td>
</tr>
<tr>
<td>Skin</td>
<td>0 out of 10</td>
<td>5 out of 10</td>
<td>0.025–0.011</td>
<td>1 × 10^3–1 × 10^3</td>
</tr>
<tr>
<td>External auditory meatus</td>
<td>0 out of 16</td>
<td>16 out of 16</td>
<td>&lt;0.010</td>
<td>1 × 10^5–5 × 10^6</td>
</tr>
<tr>
<td>Any surface</td>
<td>0 out of 16</td>
<td>16 out of 16</td>
<td>&lt;0.010</td>
<td></td>
</tr>
</tbody>
</table>

### TABLE 2
**P. AERUGINOSA IN THE DIVING HYPERBARIC COMPLEX DURING A DEEP DIVE**

<table>
<thead>
<tr>
<th>Sampling Site</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Clean washing water</td>
<td>10⁴</td>
</tr>
<tr>
<td>Bathing room floor</td>
<td>0</td>
</tr>
<tr>
<td>Work section floor</td>
<td>0</td>
</tr>
<tr>
<td>Living section floor</td>
<td>0</td>
</tr>
</tbody>
</table>

*CFU/swab
### TABLE 3
**Reservoirs of *P. aeruginosa* in a DDHC**

<table>
<thead>
<tr>
<th>Reservoir</th>
<th>Contamination Level, CFU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh water before filling the tank</td>
<td>$(2-3) \times 10^4$/ml</td>
</tr>
<tr>
<td>Washing water in the tank</td>
<td>$(2-4) \times 10^4$/ml</td>
</tr>
<tr>
<td>Internal walls of the tank</td>
<td>up to $(4-50) \times 10^4$ per swab</td>
</tr>
<tr>
<td>Water supply system tubes and terminals</td>
<td>$3 \times 10^2$ per swab</td>
</tr>
<tr>
<td>Bathing room floor</td>
<td>$(1-600) \times 10^4$ per swab</td>
</tr>
<tr>
<td>Microphone</td>
<td>up to $8 \times 10^2$ per swab</td>
</tr>
<tr>
<td>Divers’ masks</td>
<td>$(3-400) \times 10^2$ per swab</td>
</tr>
<tr>
<td>Divers’ boots</td>
<td>$(1-2) \times 10^4$ per swab</td>
</tr>
<tr>
<td>Water closet</td>
<td>$(1-4) \times 10^3$ per swab</td>
</tr>
</tbody>
</table>

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**Fig. 1.** Colonization level of *P. aeruginosa* vs. duration of volunteers’ presence in hyperbaric chamber. *a*, nose cavity; *b*, throat and mouth cavities; *c*, skin; *d*, external auditory meatus; *e*, all surfaces.

Production, one of the main factors for invasion, penetration, and spread (Table 5). Thus, the data suggest that the hyperbaric medium supports selection of a certain group of microorganisms, GNRs, which have ecological advantages in such conditions.
A DDHC may be observed as a specific niche for harmful microorganism colonization and development, particularly for *P. aeruginosa*. During more than 4 yr we have isolated identical strains belonging to one serotype—07 and the same pyocinotype from the same DDHC. Several crews have rotated through the DDHC, and different regimes and diving conditions have been established, but *P. aeruginosa* strain of the above biotype spread and colonized among crew members.

A practical aspect of the problem concerns the probable reservoirs of *P. aeruginosa* and other GNR potential pathogens in the DDHC. Tables 2 and 3 show that the most significant contamination levels in the DDHC were recorded on wet surfaces and in bathing areas. Moreover, at Day 0 of several experiments, *P. aeruginosa* was isolated from bathing water, sampled from the DDHC water supply system, tubes and from the water reservoirs. The DDHC water supply system is completely isolated from the outside during saturation. The literature reports that *P. aeruginosa* usually develops on the border between liquid and solid phases (8–10). As a result of cell adhesion, a specific biofilm is formed containing bacteria and *P. glycocalex*, a lipoprotein complex produced by the bacteria. Organic and inorganic components from the water are also involved in the glycocalyx structure. The film becomes firm and impenetrable for bactericidal agents and by antibiotics, which probably destroy only unfixed or “swimming” cells. Bacteria contained in the biofilm are preserved and can recontaminate the liquid media. To confirm the latter statement, we studied the microbial content of *P. aeruginosa*-contaminated fragments of the plastic tube of the DDHC’s water supply. The fragments were dried and then immersed in physiologic solution for 30 days, and during that period *P. aeruginosa* cells were isolated from the tube surface.

Based on these results, we consider one of the main tasks in preventing infection in a DDHC is to develop methods for decontamination, especially for the water
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supply system. These data also stress the need to prevent contamination of divers’ skin, ears, and mucous membranes, and to develop the deep diver’s resistance to infections.

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To the Editor:

Simulation of a microbial epidemic on a diving ship

Divers who work in harbors and near sewer outlets often are at risk of contamination by infectious agents, followed by development of infectious diseases (1, 2). Studies confirm the danger to divers during their contact with contaminated water, in spite of protective features of the divers’ equipment (3–5).

The literature suggests that Escherichia coli M-17 strain can serve as an indicator of epidemic processes and infectious agent transport (6). Known to be harmless to human health, the Soviet drug Kolibacterin is made from this strain.

The purpose of our work was to study the process of epidemic spread of test strain Escherichia coli M-17 among divers and crew members of ships with diving crews. The crew consists of 10 persons, including 3 divers.
<table>
<thead>
<tr>
<th>Days of Experiment</th>
<th>Engine Operator</th>
<th>Cook</th>
<th>Divers</th>
<th>Living Rooms</th>
<th>Deck</th>
<th>Water Closet</th>
<th>Kitchen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>x</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>x</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>x</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>x</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>x</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*0 — no test strain detected, no studies provided.*

*x — the sample contains test strain.*
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External surfaces of divers’ clothes and equipment, suits, boots, weights, and signal belts were contaminated with test strain fresh broth culture, diluted to a concentration of 10 colony forming units/ml. All the contaminated equipment was put on the divers, who then imitated underwater work in a freshwater lake. The supporting team members took the divers aboard from the water and helped them to undress. They then washed the divers’ equipment and clothes with soap and disinfectant.

Bacteriologic samples were obtained before the study and then daily. Samples were taken by cotton swabs from divers’ clothes, hands, and from deck equipment, kitchen tables, living and dining rooms, and door knobs. In addition, at Day 0 and at Days 6 and 20 of the investigation, intestinal flora studies of the crew were done. The test strain was identified according to its serologic features, biochemical peculiarities (saccharose reducing), and susceptibility to *Fredericq colicine*.

The data are presented in Table 1. On the day of contamination, the test strain was isolated from the divers’ clothes, from the deck floor, from knobs of deck diving mechanisms, from boots and hands of supporting divers, from the divers’ living-room door knobs, and from bathroom door knobs. However, by Days 2 and 3 of the investigation we had failed to isolate the test strain anywhere.

On Day 6 we isolated the test strain from the intestines of the engine operator and the supporting diver. After Day 12 of the investigation positive samples obtained from living and dining rooms, kitchen, and bathroom were markedly increased. Moreover, at Day 20, three other intestinal carriers were identified: two divers and the cook.

### TABLE 2

**BACTERIAL SURVIVAL ON DIVING SHIPS (DAYS)**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Diver Suit Material</th>
<th>Metal Parts of Diver Equipment</th>
<th>Metal of Ladder</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>9</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

We have managed to imitate an epidemic spread of *E. coli* strain among the crew members of the diving ship. This strain not only appeared and settled in the intestines of half of the crew members, but also developed on furnishings and equipment. Apparently a test-strain epidemic spreads from the primary contaminated objects via crew members’ hands to crew members’ intestines and to the kitchen. Having contaminated equipment and some of the crew members’ intestines, the test strain may cycle on board the ship, involving more and more objects and more crew members as its carriers. This corresponds to data in the literature, where the possibility of exogenous spread of *E. coli* among the isolated individuals was shown (7). The situation may be more dangerous the more pathogenic the specimen circulating on board the ship, not only because of the grade of its pathogenicity but also on account of its ability to stay longer on the surface of metallic and nonmetallic objects (Table 2). Thus, we speculate that colonization and spread of *Salmonella* rods on the diving
ship are more intensive than for *E. coli*, because the former may stay on contaminated surfaces for a long time, so *S. typhymurium*-contaminated surfaces may serve as additional sources of infection longer than surfaces contaminated with *E. coli*.

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To the Editor

Decompression sickness risk in women

The incidence of decompression sickness (DCS) in women remains controversial. The early work of Bangassar and Bassett has been challenged by Zwingleberg et al. (1) who claim that there is no increased incidence of DCS in women. Robinson (2) disputes this finding, on statistical grounds, and asserts that the incidence rate of DCS in women could still be between 0 and 3 times that of men. However, there is another aspect to this question. Although the question of incidence has been investigated, there has presently been no research into whether women are more susceptible to type 2 than men.

I have examined 111 cases of DCS treated at the recompression unit at the Royal Australian Navy base at HMAS Stirling. Using linear logistic regression, the association between sex and disease type was considered. From this study, women have a 4.3 times greater risk of having type 2 DCS than men (95% confidence intervals: 1.2–15.8, *P* < 0.05). There was no significant association between the variable of age, diving experience, compliance with recognized dive tables, absence of diving qualifications, or repetitive diving practices and type of disease.

Zwingleberg et al. (1) postulate that the increased incidence of DCS in women may be due to their increased adiposity when compared to men. Cole (3) notes that women
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may have increased clotting tendency, irrespective of the contraceptive pill, as well as differences in blood flow through adipose tissue, which may impede inert gas transfer. These physiologic mechanisms, while possibly contributing to an increased incidence of DCS as claimed (1, 3), may increase the risk of type II DCS in women. Further research into this area needs to look at the different susceptibilities of males and females to serious DCS as well as looking at the incidence rates and the pathophysiologic mechanisms involved.

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