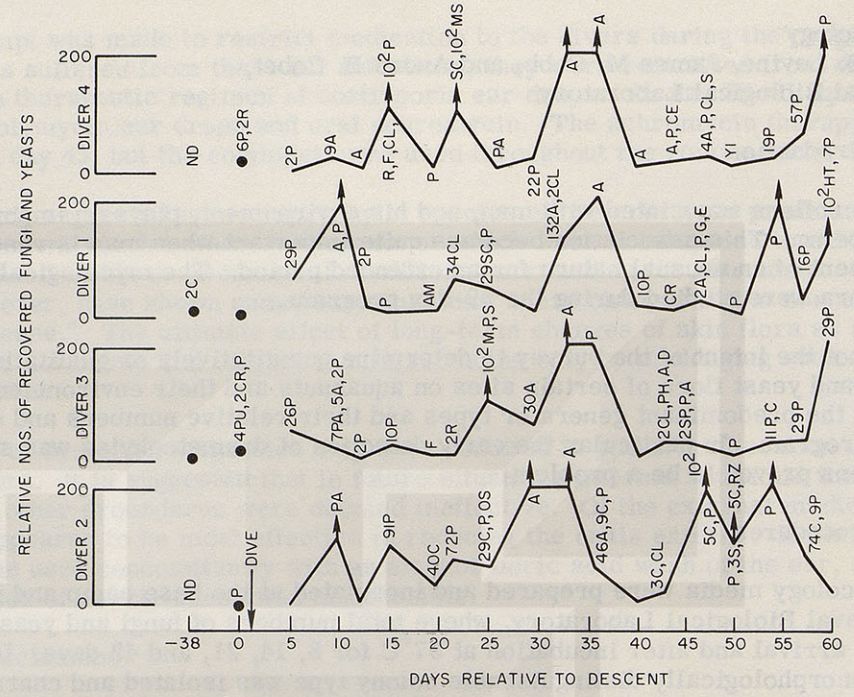


Fig. A34 - Recovery of mycoflora from throat samples. (Table A29 is a legend for the abbreviations.)

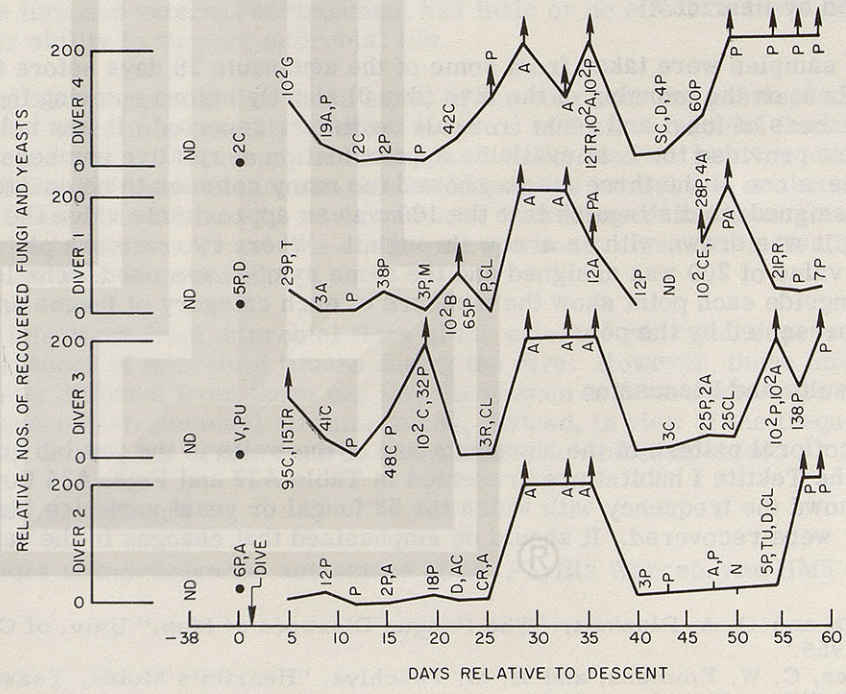


Fig. A35 - Recovery of mycoflora from ear samples

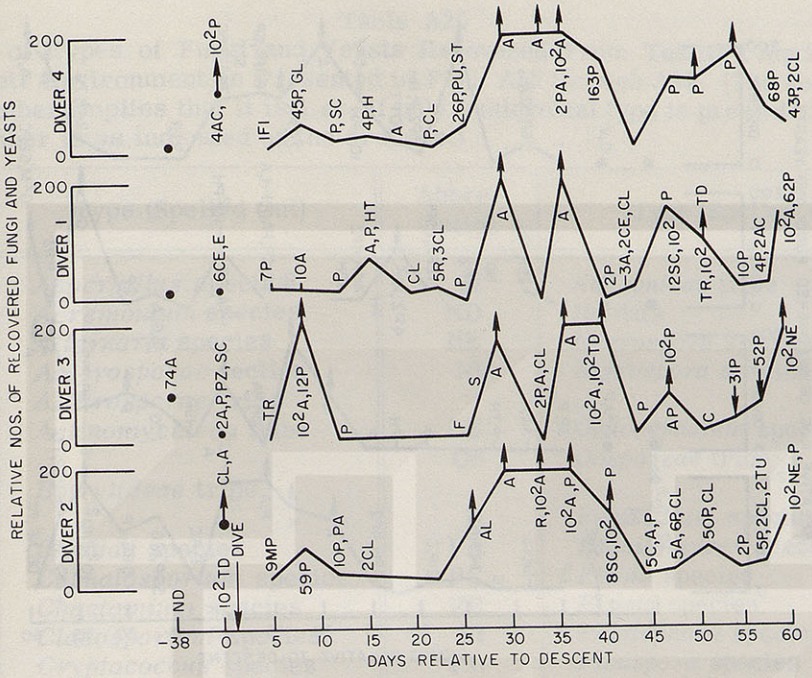


Fig. A36 - Recovery of mycoflora from skin (forearm) samples

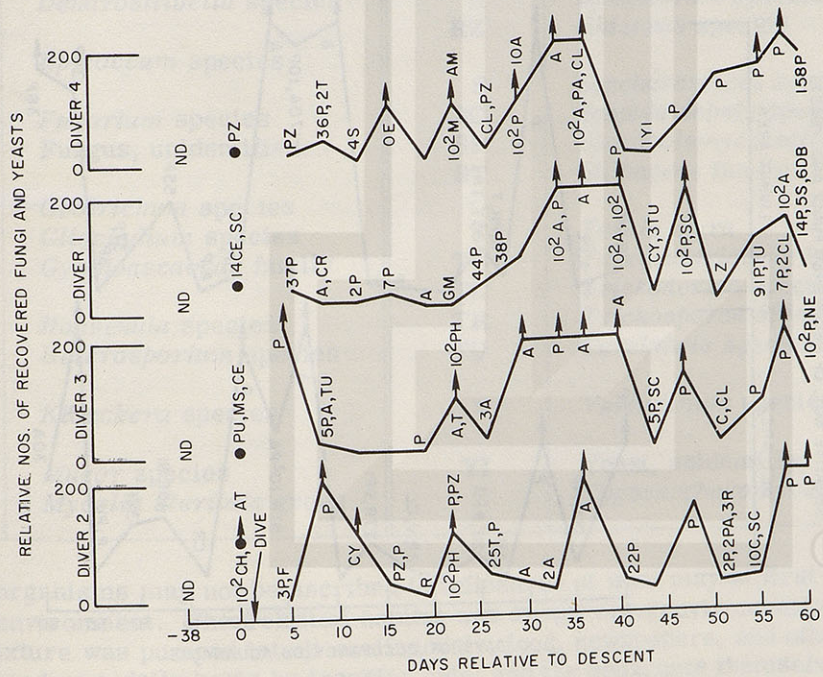


Fig. A37 - Recovery of mycoflora from skin (back of knee) samples

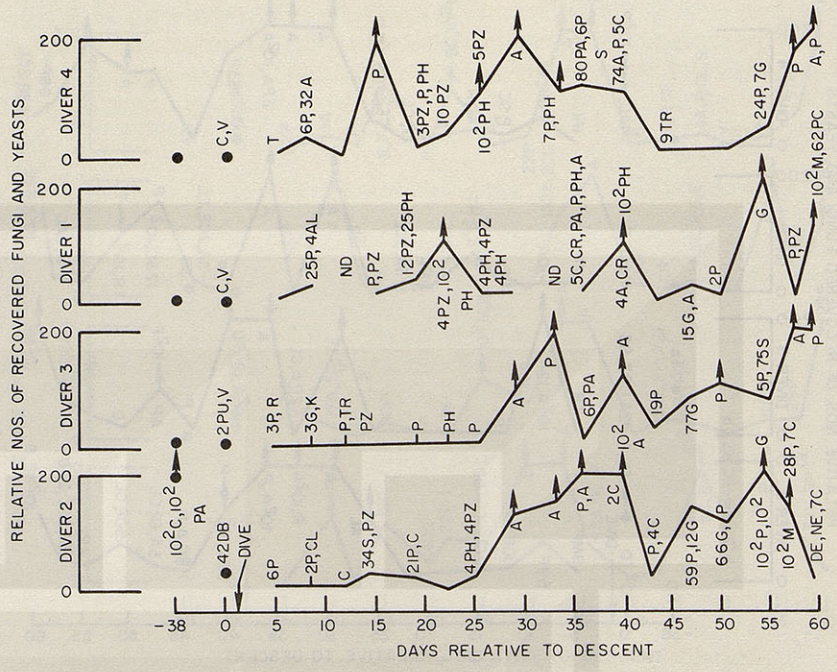


Fig. A38 - Recovery of mycoflora from rectum samples

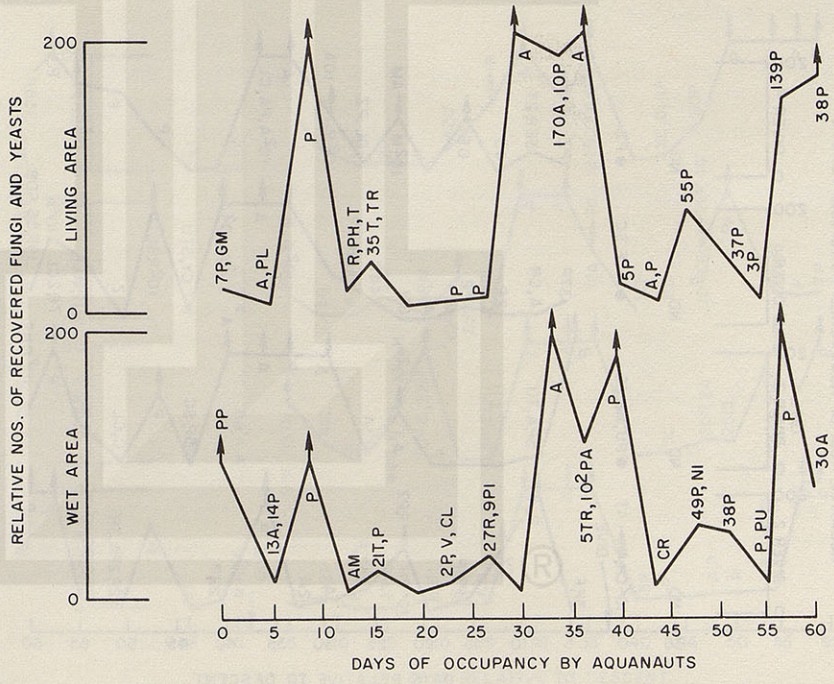


Fig. A39 - Recovery of mycoflora from habitat wall samples

Table A29
Legend of Types of Fungi and Yeasts Recovered From Tektite I Aquanauts and Their Environment as Presented in Figs. A35 through A39. (The absence of a number implies that it is 1 or, if only 1 microbial type is presented, that the number is as indicated on the ordinate.)

| Abbreviation | Type (Spelled Out) | Abbreviation | Type (Spelled Out) |
|---|---|---|---|
| A | *Aspergillus* species | N | *Nadsonieae* tribe |
| AC | *Acremonium* species | ND | No data |
| AL | *Alternaria* species | NE | *Neurospora* species |
| AM | *Amerosporae* section | NI | *Nigrospora* species |
| AP | *Aleurosporae* tribe | | |
| AT | *Actinomyceteae* tribe | OE | *Oedocephalum* species |
| | | OS | *Oosporeae* tribe |
| B | *Botrytideae* tribe | | |
| | | P | *Penicillium* species |
| C | *Candida* species | PA | *Paecilomyces* species |
| CE | *Cephalosporium* species | PC | *Pichia* species |
| CH | *Chaetomium* species | PH | *Phoma* species |
| CL | *Cladosporium* species | PI | *Phialophora* species |
| CR | *Cryptococcus* species | PL | *Pleospora* species |
| CY | *Chrysosporium* species | PP | *Papularia* species |
| | | PU | *Pullularia* species |
| D | *Dematium* species | PZ | *Plenozythia* species |
| DB | *Debaryomyces* species | | |
| DE | *Dendrostilbella* species | R | *Rhodotorula* species |
| | | RZ | *Rhizopus* species |
| E | *Epicoccum* species | | |
| | | S | *Saccharomyces* species |
| F | *Fusarium* species | SC | *Scopulariopsis* species |
| FI | Fungus, unidentified | SP | *Sporobolomycetaceae* family |
| | | ST | *Stilbaceae* family |
| G | *Geotrichum* species | | |
| GL | *Gliocladium* species | T | *Torula nigra* |
| GM | *Gymnoascaceae* family | TB | *Tuberculariaceae* family |
| | | TD | *Trichoderma* species |
| H | *Hansenula* species | TR | *Trichosporon* species |
| HT | *Heterosporium* species | TU | *Torulopsis* species |
| K | *Kloeckera* species | V | *Verticillium* species |
| M | *Mucor* species | YI | Yeast, unidentified |
| MS | *Mycelia sterilata* group | Z | *Zygosaccharomyces* species |

numbers of organisms may not be ascribed to influences of what may at first appear to be a closed environment. The Tektite I habitat was a very open environment: an unfiltered gas mixture was pumped into it continuously; food, newspapers, and other items were introduced on a daily basis by transfer pots; and the aquanauts themselves were free to enter and return from the marine environment surrounding the habitat at frequent intervals.

Thus the particularly high numbers of *Aspergillus* and *Penicillium* spores dominating almost all samples of days 26 to 36 may not be referable necessarily to population

Table A30
Frequency of Recovery of Fungi and Yeasts as a Function of Submersion Time From all Loci of Aquanauts

| Organism | Number of Times Recovered* | | | | | |
|---|---|---|---|---|---|---|
| | Total | Days 5, 8, 12, 15 | Days 19, 22, 26, 29 | Days 33, 36, 40, 43 | Days 47, 50, 54, 57 | Day 59 |
| *Penicillium* species | 191 | 48 | 30 | 29 | 67 | 17 |
| *Aspergillus* species | 94 | 13 | 22 | 46 | 10 | 3 |
| *Cladosporium* species | 26 | 3 | 7 | 5 | 9 | 2 |
| *Candida* species | 26 | 5 | 4 | 7 | 8 | 2 |
| *Plenozythia* species | 15 | 5 | 9 | 0 | 1 | 0 |
| *Phoma* species | 14 | 0 | 10 | 4 | 0 | 0 |
| *Geotrichum* species | 10 | 2 | 0 | 0 | 8 | 0 |
| *Paecilomyces* species | 14 | 3 | 1 | 8 | 2 | 0 |
| *Saccharomyces* species | 9 | 2 | 2 | 1 | 3 | 1 |
| *Scopulariopsis* species | 9 | 1 | 2 | 3 | 3 | 0 |
| *Rhodotorula* species | 7 | 2 | 3 | 0 | 2 | 0 |
| *Mucor* species | 6 | 2 | 2 | 0 | 1 | 1 |
| *Trichosporon* species | 6 | 3 | 0 | 2 | 1 | 0 |
| *Torulopsis* species | 5 | 1 | 6 | 0 | 4 | 0 |
| *Torula nigra* | 5 | 3 | 2 | 0 | 0 | 0 |
| Others† | < 5 | | | | | |

*Values show number of samples that were positive without regard for the number of organisms recovered from each positive sample.
†See Table A29.

dynamics originating within the habitat. The organisms could have been introduced via the air or, unknowingly, by the introduction of food, such as fruit, that soon underwent fungal spoilage. In this regard the high numbers that were detected in the living quarters on day 29 were detected in only the wet lab on the next sampling occasion, which was day 33 of the dive (Fig. A39).

Of considerable interest was the consistency with which the elevated numbers of *Aspergillus* and *Penicillium* of days 29 to 36 were recovered. The organisms were found in all of the aquanauts (at virtually all of the sampling loci) and on the walls of the habitat. This observation suggests that the methods employed were adequate to detect gross population changes and that any tendency for certain varieties of fungi or yeast to become established preferentially in the habitat would have been detected.

It was anticipated that the wet lab, through which the aquanauts and materials gained entry into the habitat, would develop high humidity. This high humidity could then be expected to favor fungal growth. However, neither contingency occurred during the dive; the air conditioning system performed well, and the wet lab, like the rest of the habitat, showed mean temperatures of 25 to 27°C and mean relative humidity values of 52 to 54%.

A concern with possible mycologic consequences that also did not materialize pertained to skin disease. The aquanauts showered frequently and washed with a soap containing hexachlorophene; they were little bothered with skin disease. On day 7 of the dive aquanaut 2 showed skin irritation on the lower anterior portion of the neck. This was attributed to a close fitting nylon collar on his underwater clothing.

Irritations of the ear, however, did present a continuing problem beginning on day 11 of the dive. The resulting otitis externa in all of the divers appears to have been entirely of bacterial origin, as no mycological basis was found for the infection.

On different occasions some of the aquanauts experienced sore throats and diarrhea. Neither of these disorders was associated with remarkable changes in fungal or yeast numbers or varieties. Aquanaut 2 yielded a *Candida* species from the throat or rectum on numerous occasions; however, he was not afflicted with either throat or bowel disorders.

Aquanaut 2 first experienced ear symptoms (slight squeeze, left ear) on day 11 of the dive, and both ears showed this symptom on day 15. He also showed slight inflammation of the right canal on day 26, and his medical status report shows that both ears were "infected" on day 33. He was treated with cortisporin and tetracycline beginning on day 36. *Candida* was first isolated from his throat on day 20 and, again, on days 26 and 36. It does not appear to have been potentiated by the antibacterial treatment, and the data are insufficient to ascribe an etiologic or commensal role to it in the ear infection.

At the completion of the program rug samples were removed from various localities in the habitat and studied for the presence of fungi (Fig. A40). The results are not

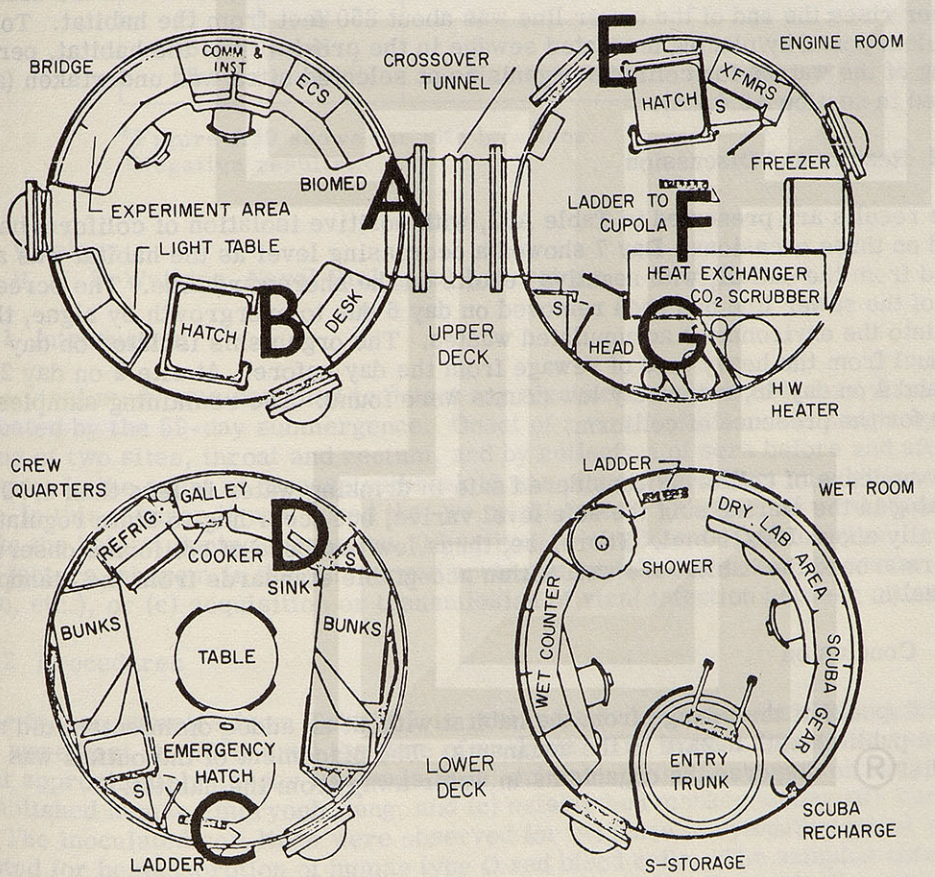


Fig. A40 - Localities from which rug samples were removed and studied for fungi. The organisms found were Penicillium ($1.5 \times 10^2$ organisms/g at site A and 5.1 organisms/g at site F), Rhodotorula (4.5 organisms/g at site G), and Mucor (6.9 organisms/g at site C and $4.0 \times 10^7$ at site D). No organisms were recovered at sites B and E.

remarkable with the exception of the high number of *Mucor* (a common bread mold) at site D. This is in front of the galley area and most probably results from food and bread crumbs dropped in that area. The fungi isolated from the sea-water samples were the ubiquitous saprophytes typically found in nature.

A3.4.6.4 Conclusion

The varieties of organisms isolated from those aboard the habitat (Table A30) and from the structure itself (Figs. A39 and A40) do not appear to be unusual. The absence of fungus- or yeast-related disease among the divers also suggests that the habitat did not present a mycologically stressful situation.

A3.4.7 Marine Microbiology
Andre B. Cobet, Naval Biological Laboratory

A3.4.7.1 Introduction

The sewage from the habitat was macerated, piped through 1000 feet of 4-inch hose, and disposed into the marine environment. Due to the bottom contour and the desire not to go over rises the end of the sewer line was about 850 feet from the habitat. To assess the problem posed by placing untreated sewage in the proximity of the habitat, periodic sampling of the waters for coliform organisms at selected sites was undertaken (as was described in section A3.4.2.4).

A3.4.7.2 Results and Discussion

The results are presented in Table A31, with positive isolation of coliform bacteria obtained on three occasions. Day 7 showed a decreasing level as the habitat was approached from the outfall, with negative results on the shoreward side. The screen at the end of the sewer line had been removed on day 6 due to overgrowth by algae, thus releasing into the environment accumulated wastes. The organisms isolated on day 7 may be residual from the heavy load of sewage from the day before. At site 2 on day 20 and sites 2 and 4 on day 48, extremely low counts were found. The remaining samples were negative for the presence of coliform.

The numbers of coliforms considered safe in drinking water is less than 4/100 ml.* For bathing in the marine surf the safe level varies, but according to State regulations it is generally above 100/100 ml. Therefore, these low numbers of coliforms observed in the waters around the habitat are well within acceptable standards from the standpoint of public health.

A3.4.7.3 Conclusion

The disposal of the sewage from the habitat without an added disinfectant did not produce a public health hazard to the aquanauts. The placement of the outfall was sufficiently distant to disperse the organisms in water away from the habitat.

*"Standard Methods for the Examination of Water and Wastewater," 12th edition, Amer. Pub. Health Assoc. Inc., New York, pp. 569-570, 1965.

Table A31
Quantity of *Coliform* Organisms per 100 ml of Sea Water Collected at Four Sites* in the Area of the Habitat

| Day of Dive | Quantity of Organisms (count/100 ml) | | | |
|---|---|---|---|---|
| | Site 1 | Site 2 | Site 3 | Site 4 |
| 0 | N† | N | N | N |
| 3 | N | N | N | N |
| 7 | 2 | 3 | 6 | N |
| 9 | N | N | N | N |
| 13 | N | N | N | N |
| 16 | N | N | N | N |
| 20 | N | 1 | N | N |
| 23 | N | N | N | N |
| 30 | N | N | N | N |
| 35 | N | N | N | N |
| 41 | N | N | N | N |
| 48 | N | 3 | N | 2 |
| 55 | N | N | N | N |

*Figure A30 shows the site locations.
†Negative results.

A3.4.8 Virology
H. M. S. Watkins, Naval Biological Laboratory

A3.4.8.1 Introduction

The viral study was designed to detect any significant impact of human viral agents exacerbated by the 59-day submergence. Onset of a viral infection was sought by regular sampling of two sites, throat and rectum, and by collection of sera before and after submergence. In the event of disease outbreak it was planned to collect an additional acute serum. In this manner it was hoped that we might detect: (a) onset of viral disease carried into the habitat during incubation, (b) activation of a latent viral infection by environmental factors inherent in the submerged environment (altered stress, pressure, gas mixture, etc.), or (c) acquisition or transmission of viral infection between aquanauts.

A3.4.8.2 Procedures

The frozen samples from Tektite I, in veal infusion broth and the CRTV holding media, were kept at -70°C until the time of analysis. The thawed specimens were passed twice at approximately 14-day intervals in (a) primary human embryonic kidney cells, (b) established human embryonic lung, and (c) established monkey embryonic kidney cell lines. The inoculated cell lines were observed for evidence of cytopathological effects and tested for hemadsorption of human type O red blood cells. The samples obtained during the stay of aquanauts at the University of Pennsylvania hospital, the oyster samples, and a throat sample from diver 7 collected during the onset of the first respiratory infection following completion of the trial were passed three times as outlined.

### A3.4.8.3 Results and Discussion

The specimens collected in the base-line studies at the University of Pennsylvania and at the Tektite I site were all negative for cytopathological effects and hemadsorption. No virus was demonstrated in the samples, and no clinical viral disease was reported during the study.

### A3.4.8.4 Conclusion

The conditions imposed by environmental changes inherent in the Tektite I submergence did not activate a latent virus infection, nor did the aquanauts acquire a demonstrable virus infection from the marine environment.

## A3.4.9 Nasal *Staphylococcus* "Carrier"

Andre B. Cobet and John Hresko, Naval Biological Laboratory

### A3.4.9.1 Introduction

The carrier of a pathogenic organism living in close association with other people presents a potential hazard to his associates. The degree of hazard depends on such conditions as the type of organism,* degree of crowding, and number of susceptibles,† on environmental factors such as relative humidity and the degree and type of lighting,‡ on the temperature of the environment,§ and on other factors.

The association of the four aquanauts in the Tektite I environment provided an opportunity to study the transmission of a tracer organism. None of the aquanauts were carriers of *Neisseria meningitidis*; however, both aquanauts 2 and 4 were nasal carriers of coagulase-positive *Staphylococcus aureus*.

### A3.4.9.2 Procedure

The sample swab was obtained by the aquanaut placing a dry sterile swab high into the nose. Isolation of nasal *Staphylococcus aureus* was made by streaking the surface of a plate of mannitol salt agar (Difco) with the nasal swab and incubating the inoculated plate at 37°C for 24 hours. Colonies which fermented mannitol were subcultured and returned to NBL for identification by standard methods of identification.

Specimens were obtained from all aquanauts on days 22, 40, and 59.

### A3.4.9.3 Results and Discussion

A carrier state was found to exist in aquanauts 2 and 4 at the beginning of the program. Aquanaut 2 on subsequent analysis did not yield the nasal *Staphylococcus*. Aquanaut 4 was found to be a carrier through day 40 but not on day 59. The other two aquanauts were not found to carry nasal *Staphylococcus* on the sampling days.

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*P. H. Gregory and T. L. Monteith, editors, "Airborne Microbes," Cambridge University Press, England, 1967.

†L. F. Miller, "Acute Respiratory Infections in Naval Personnel," pp. 3-23 in "A Symposium on Aerobiology," Naval Biological Laboratory, Naval Supply Center, Oakland, 1963.

‡S. J. Webb, "Factors Affecting the Viability of Airborne Bacteria, IV - The Inactivation and Reactivation of Airborne Serratia marscescens by Ultraviolet and Viable Light," Can. J. Microbiol. 7, 607-619 (1961).

§D. N. Wright, G. D. Bailey, and L. J. Goldberg, "Effect of Temperature on Survival of Airborne Mycoplasma pneumoniae," J. Bacteriol. 99 (1969).

The close association of the aquanauts and environmental factors were not optimal for the transmission of *Staphylococcus aureus* from the two carriers to the two noncarriers. At the completion of the 59-day program there were no nasal *Staphylococcus aureus* carriers remaining. The similar loss of the carrier state has been demonstrated by other investigators.

### A3.4.9.4 Conclusion

The two aquanauts who were nasal *Staphylococcus* carriers at the beginning of the program had lost their carrier state when examined at intervals during the 59-day study. The two noncarriers remained uninfected.

## A3.4.10 General Discussion of Results and Conclusions

Andre B. Cobet and John P. Hresko, Naval Biological Laboratory

At the beginning of the Tektite I program several questions were unanswered concerning the microbiology associated with a prolonged saturation dive. These were questions such as: Would the microbial population in the environment build up during the program and, if so, in what manner? Would changes occur within the normal microflora of the aquanauts? Would organisms indigenous to the aquanauts and the environment, both habitat and marine, present a health problem? Were the aquanauts healthy carriers of potentially pathogenic organisms, and, if so, would transmission to other aquanauts occur? What degree of health hazard would be present from the disposal of untreated sewage into the marine environment? Would the prolonged application of the conditions necessary to maintain the Tektite I program (increased pressure, altered atmosphere, etc.) result in a change in the normal relationship between the aquanaut and his microflora? Only a comprehensive study could give answers to these questions.

There were certain correlations in the data collected in the various sections, particularly between aerobiology, bacteriology, and mycology.

During the latter half of the program the *Acinetobacter*, which was frequently isolated from the air samples and samples from the skins of the aquanauts, may have had its origin from the marine environment. This is based on the following evidence. The aquanauts appeared to have acquired the organism after the start of the program, as all samples were negative for this organism before entry in the habitat. Aquanauts 2 and 3 were first to demonstrate the organism in samples from the skin, followed on days 19 and 22, when the two other aquanauts demonstrated the organism. They could have acquired the organism directly from the marine environment or from the aerosol resulting from the activity of the other aquanauts. The marine agar showed a relatively constant background of organisms until day 33, at which point the level rapidly increased. From day 33 to day 38 at least two of the aquanauts were restricted to the habitat at any one time due to ear infections. During this period of restriction there was a reduction in frequency of showering, which allowed for a buildup of population on the skin. An increase in the in-house activity resulted in shedding of the organism and consequently aerosolization. This would be reflected as an increase in frequency of isolation of the particular organism from the air and skin samples which was found. The *Acinetobacter* had established itself in the skin flora of all four aquanauts midway through the program. That the organisms originated from the aquanauts and their activity is demonstrated by the decrease in the number of organisms in the air 48 hours after the exit of the aquanauts from the habitat to $0.3/ft^3$. During this 48 hours there was no activity in the habitat.

Potentially pathogenic organisms were isolated from the air during the program; had they become established in the air in large numbers, the health of the aquanauts could have been jeopardized.