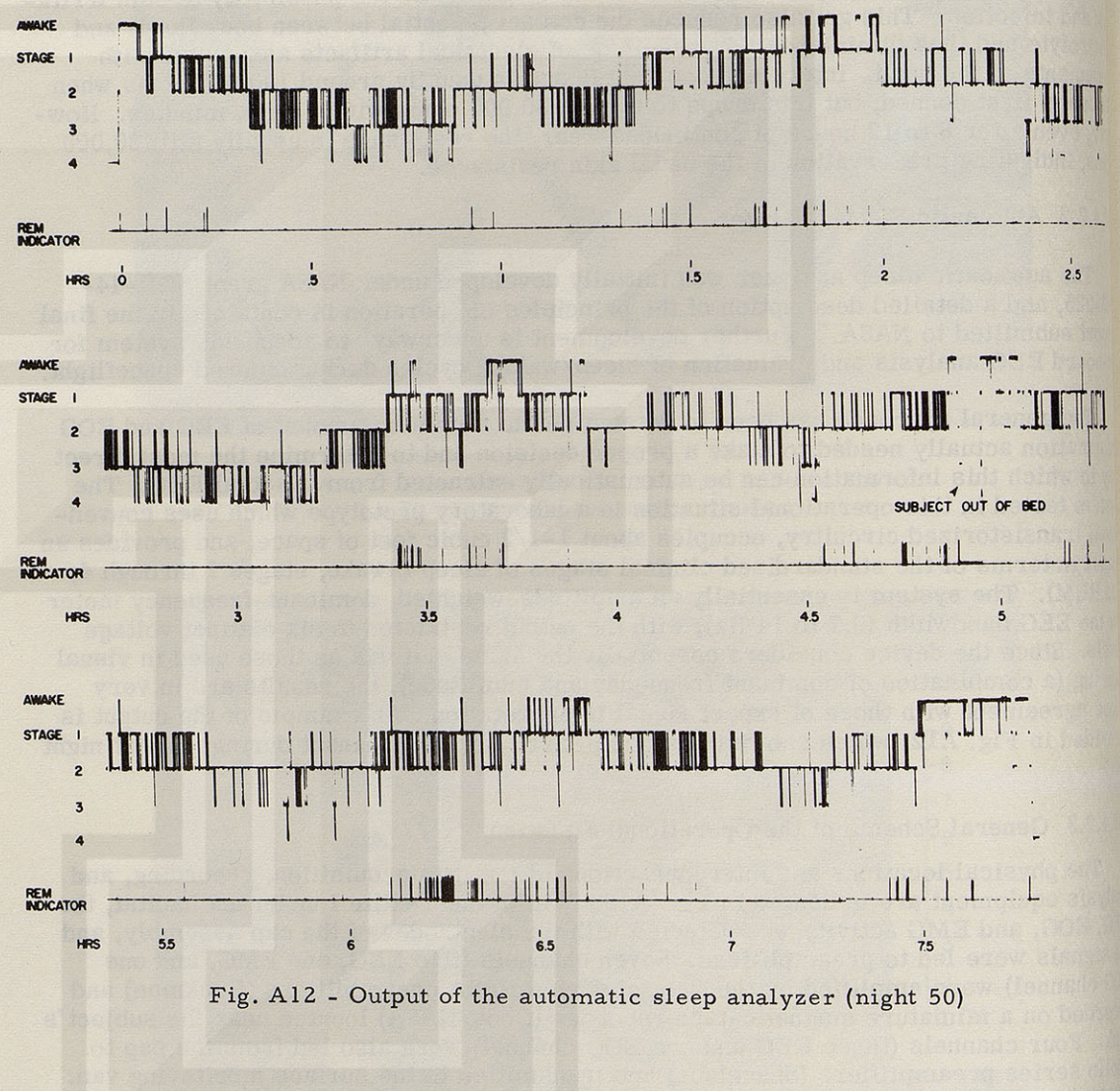


Fig. A12 - Output of the automatic sleep analyzer (night 50)

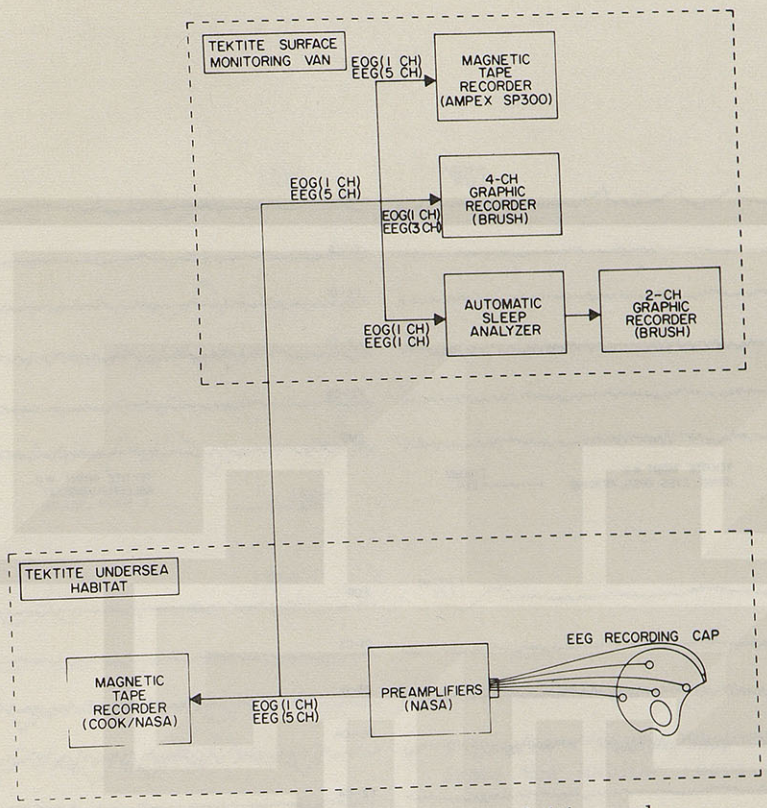


Fig. A13 - Automatic EEG acquisition and data-analysis equipment used during the Tektite I project

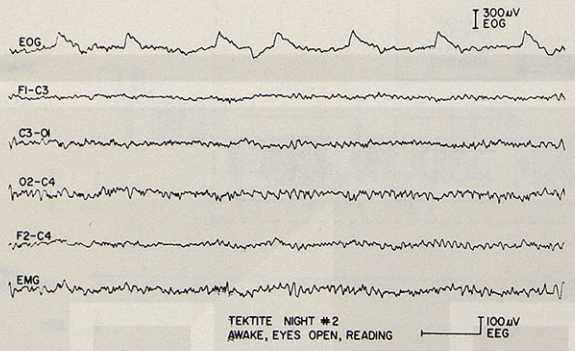
The performance of the automatic analyzer was constantly evaluated throughout the sleep period by a human electroencephalographer who observed the EEG and EOG on the four-channel graphic recorder and noted any areas of disagreement or any type of artifactual activity which might influence the results.

A2.4.3 Results

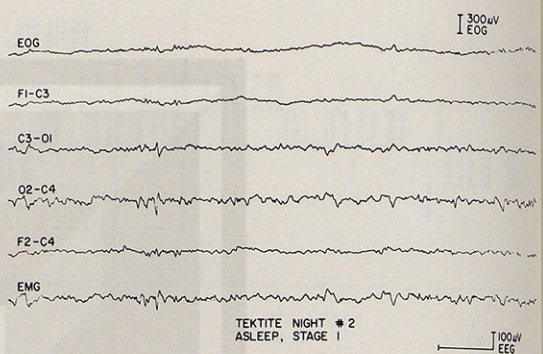
A2.4.3.1 General

In general all components of the system performed well, and no problems were encountered which could be attributed to specific environmental conditions. In spite of the fact that recording was carried out in an unshielded bunk in close proximity to other electrical apparatus, electrical interference was never serious. Data were lost through equipment failure during only one recording period, recording night 51, when both the habitat recorder and surface equipment detected only random-appearing electrical noise. Although the reason could not be established with certainty, a transient fault in the preamplifier power supply is suspected. Approximately 1-1/2 hours of recording were lost during the initial portion of night 58, when the subject retired for the night (unobserved by surface monitors) and neglected to turn on his power switch. This was corrected later by another crew member when the situation was recognized. As will be discussed, no recording was attempted on days 3 and 7.

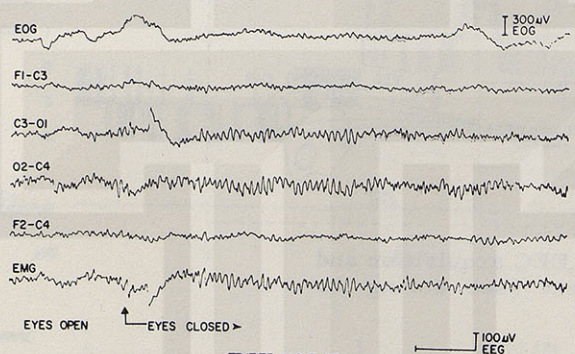
The quality of the recordings is illustrated in Fig. A14, which recordings were made by playing the tape-recorded data from the habitat recorder back through a conventional



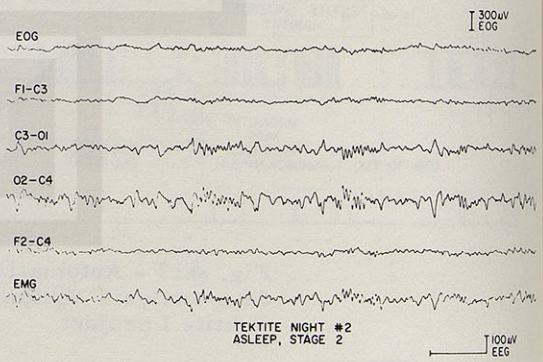
TEKTITE NIGHT #2
AWAKE, EYES OPEN, READING



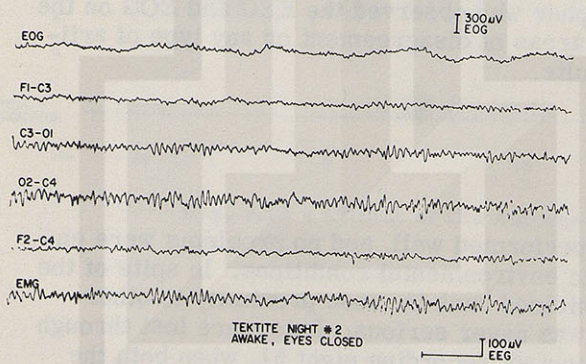
TEKTITE NIGHT #2
ASLEEP, STAGE 1



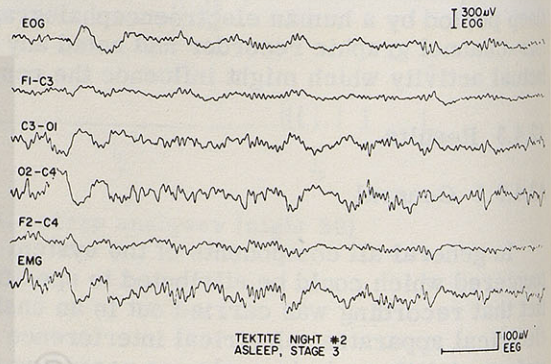
TEKTITE NIGHT #2
AWAKE, CLOSING EYES



TEKTITE NIGHT #2
ASLEEP, STAGE 2



TEKTITE NIGHT #2
AWAKE, EYES CLOSED



TEKTITE NIGHT #2
ASLEEP, STAGE 3

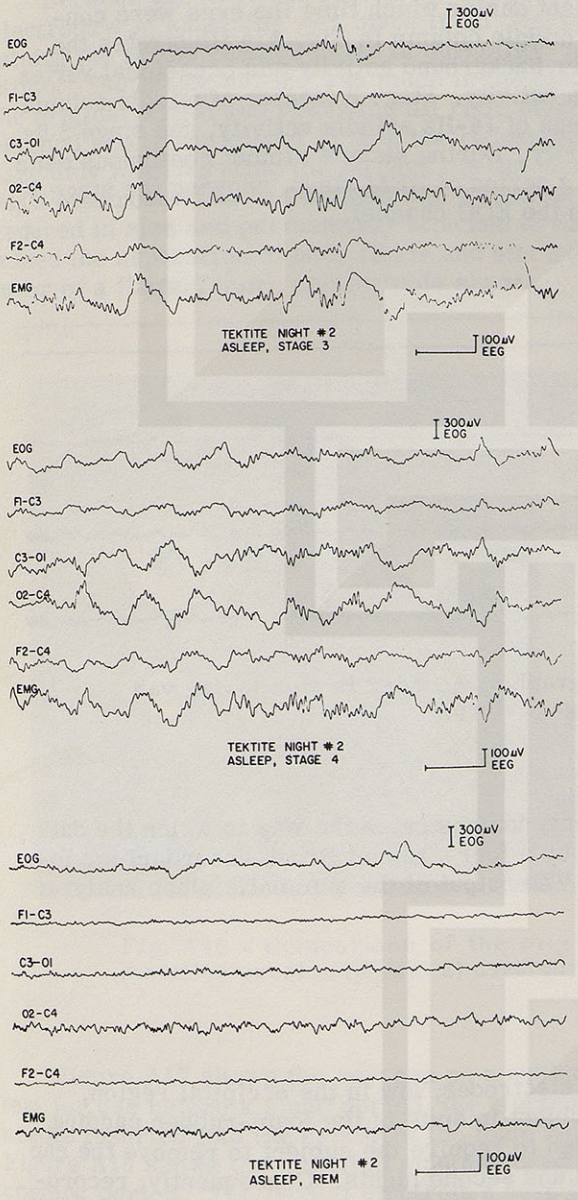


Fig. A14 - Samples of data played back from the habitat recorder (Fig. A13)

Grass EEG machine. The sample at the upper left in Fig. A14 shows the EEG pattern with the subject awake, reading in bed. The EOG channel demonstrates the typical scanning-type eye movements associated with reading. Occipital alpha activity is present intermittently in this eyes-open recording. The sample at left center shows a high-amplitude burst of alpha waves which occurs when the subject closes his eyes, and the sample at the lower left is from a long segment during which time the eyes were constantly closed. The sample at the top of the middle column in Fig. A14 illustrates the change with onset of sleep, showing the slower background activity and occasional vertex (C_3 and C_4) transient forms and lack of alpha activity. Stage 2 (center of middle column of Fig. A14) is characterized by the appearance of 14-Hz spindle activity, and stage 3 by increasing amounts of intermittent delta activity. During stage 4 almost continuous delta activity is evident. The last sample is from a period of REM sleep, showing the stage 1 EEG and occasional abrupt eye movements in the EOG channel.

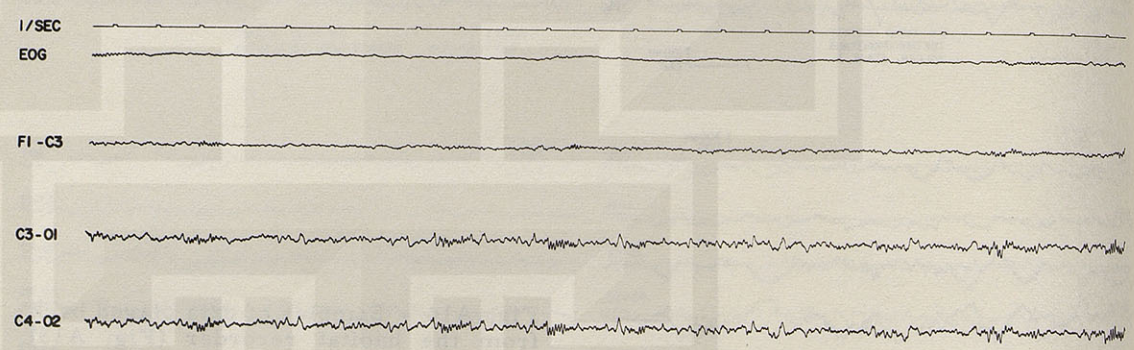


Fig. A15 - Sample of data from the graphic recorder in monitoring van (night 2, stage 2 sleep)

Figure A15, from the same recording night, demonstrates the way in which the data were displayed on-line in the monitoring van (by a four-channel Brush recorder) for interpretation by the electroencephalographer. The output of the automatic sleep analyzer was shown in Fig. A12.

A2.4.3.2 Problems Encountered

A2.4.3.2.1 Recording Cap

During the first recording night the EMG electrodes, low in the occipital region, were found to be quite uncomfortable by the subject in spite of the foam-rubber padding in this area. The discomfort was severe enough to require the subject to remove the cap before the end of the sleep periods of the first and second nights. Consequently, recording was suspended for the third night, and the caps were modified by removal of the EMG electrodes.

This only slightly improved the comfort, and the occipital electrodes were now the most bothersome, although the quality of the recordings continued to be good on nights 4, 5, and 6. Because the subject began to notice discomfort persisting on throughout the day in the area where the occipital electrodes contacted the scalp at night, recording was not carried out on night 7. The cap was tried again on night 8, but since discomfort persisted, conventional chlorided silver-disk electrodes (Grass) were substituted for the cap on the

last two nights (9 and 10) of the first 10-day recording period. These electrodes were applied by other crew members who had previously been trained in the technique of application.

Between the first and second 10-day recording periods, an extensive redesign of the electrode-cap assembly was made in an attempt to improve the comfort while still maintaining the prime requirements of durability, nonirritability, and satisfactory data acquisition. The major change was made in the electrode itself by reducing its size to 1/3 of the original length and removing all rigid plastic components. Figure A16 compares the original electrode (model 1) with the redesigned (model 2) version. The large plastic assembly incorporating the Ag/AgCl pellet electrode was eliminated, and a flatter Ag/AgCl electrode disk was molded into a flexible silicone-rubber housing. The sponge was reduced in size and permanently attached to the housing. This electrode thus compromised the separation of body fluids and electrode — a feature of the original model — in favor of a flatter, more comfortable shape.

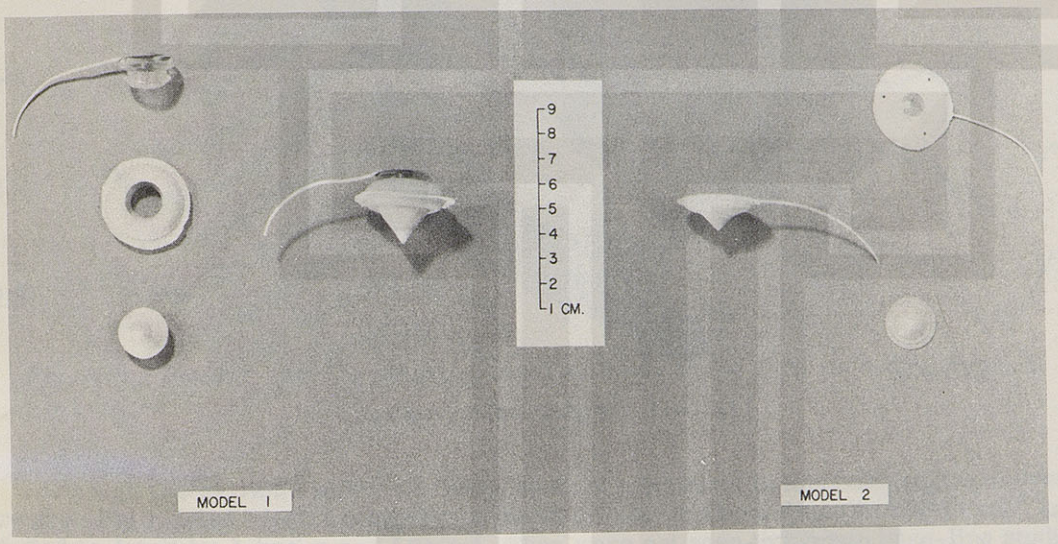


Fig. A16 - Comparison of the original electrodes (model 1) and the modified version (model 2) used during the final series of recordings

Figure A17 shows the new cap on a subject in the laboratory. In preparing this system for use before a sleep period, the electrolyte gel is injected through a hypodermic needle inserted into the center of the foam rubber until the entire sponge is saturated. Figure A18 shows the modified montage used in the model 2 cap for the final 10-day recording period. The frontal electrodes have been eliminated, since they were unnecessary for the evaluation of sleep recordings. The model 2 cap was worn by the subject during recording nights 50 through 58.

A considerable improvement in comfort was reported by the subject ("80% better"), although he did continue to experience some discomfort in the scalp areas contacted by the electrodes. During the last day he also reported the presence of swellings or bumps in the occipital areas which he felt were related to the electrodes. The quality of the recordings continued to be good, and the increased comfort permitted uninterrupted records during this final period of the project.

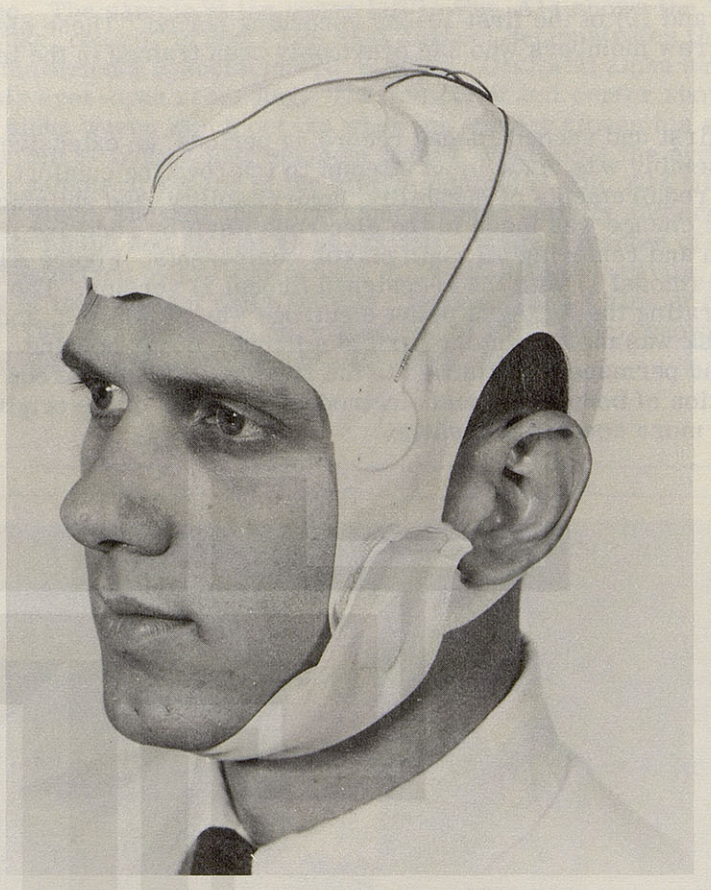


Fig. A17 - Model 2 electrode cap

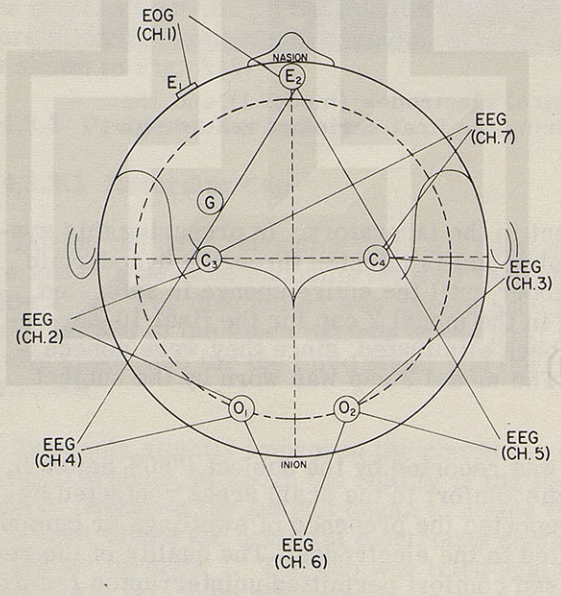


Fig. A18 - Modified montage used in the model 2 electrode cap during the final ten-day period

(Since completion of the Tektite I project, further modification has been made in the electrode-cap system to increase the comfort, and the revised model was used successfully in the Gulf Stream drift mission aboard the submerged research vessel *Benjamin Franklin*. A crew member wore this cap assembly during 15 preselected nights of the 30-day mission and did not experience either discomfort or scalp bumps.)

A2.4.3.2.2 Electrodermal Artifacts

Figure A19 illustrates a phenomenon often seen during stages 3 and 4 of sleep, occasionally during stage 2, but never in stage 1 at the onset of sleep or during REM. These high-amplitude, slow transients often occurred in long runs, becoming almost continuous, and lasting up to an hour in some cases

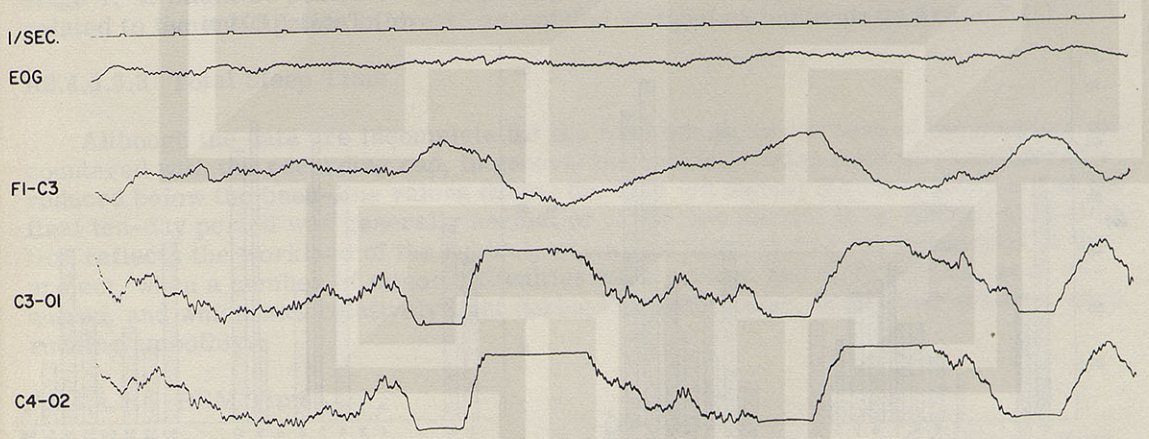


Fig. A19 - Sample showing electrodermal artifacts in the EEG recording (night 2)

Although the galvanic skin resistance was not monitored in this subject, these events are probably related electrodermal responses similar to those described by Burch* and studied in detail by Johnson and Lubin.† They were not seen in the two base-line studies of this subject; however, these two laboratory recordings were made using conventional EEG electrodes and routine skin preparation which results in low interelectrode resistance and destruction of the skin's ability to produce electrodermal responses. In contrast, the electrode cap preserves the integrity of the skin and presumably its ability to produce the responses.

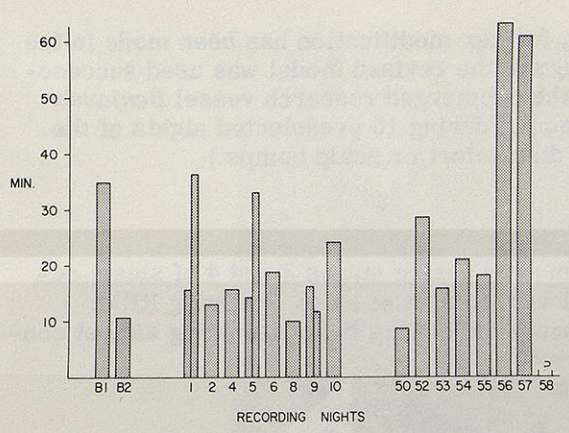
A2.4.3.3 Evaluation of Sleep Recordings

A2.4.3.3.1 Introduction

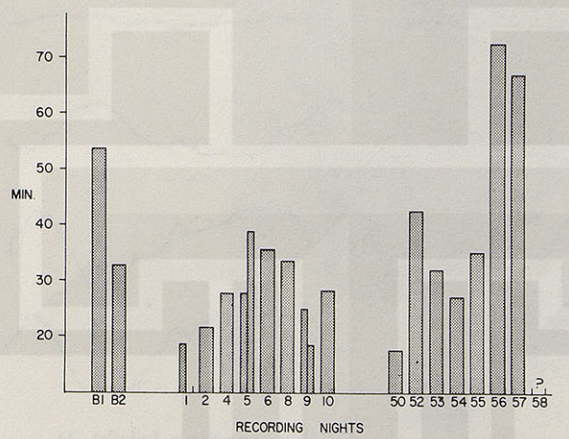
Although the significance of alterations in sleep patterns was not the primary goal of our participation in the Tektite I project, several points are worthy of mention and further

*N. Burch, "Data Processing of Psychophysiological Recordings," pp. 165-180 in "Symposium on the Analysis of Central Nervous System and Cardiovascular Data Using Computer Methods," L. D. Proctor and W. R. Adey, editors, Washington, D.C., NASA, 1965.

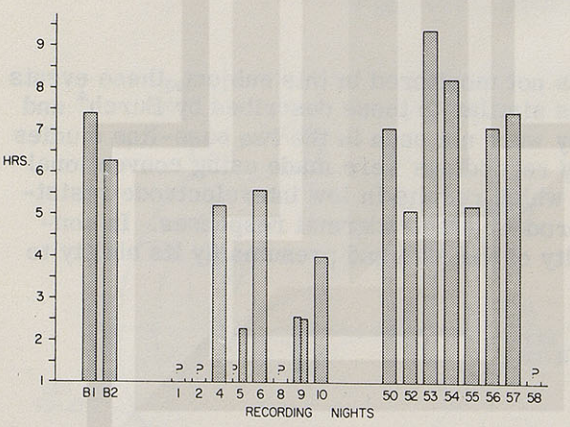
†L. C. Johnson and A. Lubin, "Spontaneous Electrodermal Activity During Waking and Sleeping," Psychophysiology 3, 8-17 (1966).



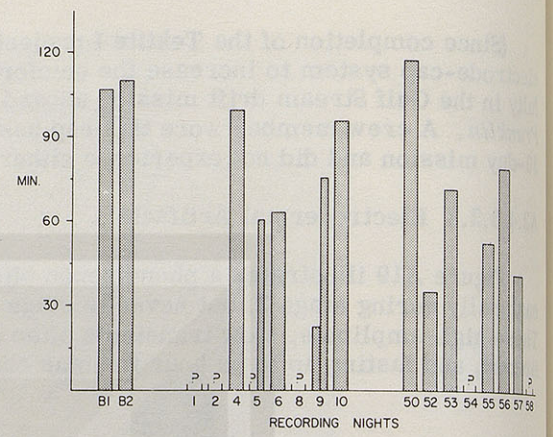
(a) Time to attain stage 2 sleep



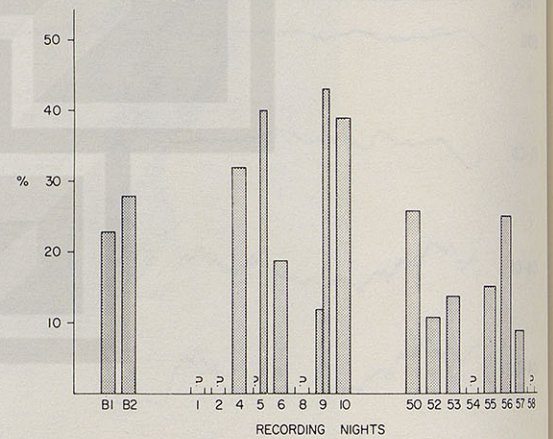
(b) Time to attain stage 4 sleep



(c) Total sleep time



(d) Total REM time



(e) Percent REM time

Fig. A20 - Comparisons of selected aspects of subject's sleep during base-line nights (B1 and B2), the first ten-day recording series (nights 1 through 10), and the final ten-day recording series (nights 50 through 58). (Split bars during the first ten nights indicate two sleep periods because of watch schedules.)

consideration. Figure A20 compares selected aspects of the subject's sleep during the first and last recording sessions with the findings during the two base-line nights (B1 and B2) spent in the laboratory.

A2.4.3.3.2 Time to Fall Asleep

Figure A20a shows the amount of time which the subject spent in bed before falling asleep for the first time. This time was measured from the point at which he was observed to get into the bunk until the first EEG signs of stage 2 sleep (vertex transients and spindles). Thus, for this measure, brief periods of drowsiness (stage 1) were included in the cumulative time.

The subject thus experienced no difficulty going to sleep during the first 10-day period, although the time does not appear to be significantly shorter than the base-line times. During the final days of recording, however (nights 56 and 57), there was a marked increase in the time before sleep onset — in both cases, exceeding an hour. This same information is also shown in Fig. A20b, which indicates the time required to reach stage 4. It might be postulated that perhaps this increase in time before sleep onset was related to the anticipation of events associated with the end of the mission.

A2.4.3.3.3 Total Sleep Time

Although the data are incomplete for the first ten nights because of the problem encountered with the recording cap, in general the subject's sleep time was considerably reduced below the base-line values during the first ten nights; the sleep time during the final ten-day period was generally normal or more than normal (Fig. A20c). This situation reflects the workload of the subject, which was heavy during the initial part of the project, when a number of minor difficulties were present and night watches were required, and which was relatively light during the final phases, when most systems were running smoothly.

A2.4.3.3.4 REM Time

Although the total sleep time was generally reduced below normal during the first ten days, as indicated in Fig. A20d there was a definite tendency for the total REM time to approach the base-line values. This effect is seen more clearly in Fig. A20e, which shows the percentage of total sleep time occupied by the REM stage. It is obvious from this figure that there was a marked increase above the base-line values in the percent REM time during many of the nights in the first 10-day session. During the final 10 days, the percent REM time was similar to or slightly below base-line values. The significance of this finding is unknown, but again it could be related to the increased workload, and perhaps stress, of the initial portion of the project.

A2.4.4 Conclusions

The operational testing situation provided by the Tektite I project led to three major conclusions or accomplishments:

1. The compatibility of all phases of the EEG acquisition and analysis system was assured, and no significant problems were encountered with respect to extraneous electrical interference.

2. The problems encountered with the electrode-cap assembly in the early phases of the mission led to extensive redesign and miniaturization of this unit. Further improvement since the end of Project Tektite I has resulted in a much more satisfactory recording cap with respect to wearer comfort and ease of application.

3. The performance of the automatic analysis system demonstrated the ability to obtain reliable information concerning the subject's quantity and quality of sleep. Because of the immediate availability of the results, this information could theoretically be used to optimally regulate the subject's next work/rest period.

A2.5 Psychomotor Performance

Rayford Saucer and Stanley Deutsch, National Aeronautics and Space Administration, Langley Research Center, Hampton, Virginia

A2.5.1 Introduction

Man, with his rapidly advancing technology, will venture farther and longer into the hostile and semihostile environment of inner and outer space to perform useful tasks. One of the major objectives of Project Tektite I was to study the ability of aquanauts to adapt to the environment and confinement in an undersea habitat and the effects on their capabilities for completing complex psychomotor tasks. The factors of confinement and isolation were assumed to be analogous to conditions that may exist in future manned space flight of similar duration.

A2.5.2 Development of Psychomotor Performance Tester

Complex psychomotor coordination was measured on the National Aeronautics and Space Administration complex coordinator developed at the Langley Research Center (LRC) by Jim Scow. The LRC complex coordinator is a human performance measurement device originally developed to measure small changes in psychophysiological functions in drug and environment studies. This test device was based on a concept developed in 1939 at the University of California for the selection of aviation cadets and studies of anoxemia.

While searching for a psychomotor test instrument that could be used in closed environments, such as space cabin simulators, it was decided to determine whether this device could provide sufficiently sensitive differences to measure slight decrements in performance as an indication of stress. The LRC complex coordinator was initially used in a 28-day chamber run in which an integrated life support system was being tested.

The LRC complex coordinator was deemed to be sufficiently reliable to warrant its use on the 60-day Tektite I mission and on the 30-day Gulf Stream mission aboard the *Benjamin Franklin*. To meet the operational requirements of these two underwater studies the test device had to have the capability for self-administration and self-scoring, requiring a built-in programmer, counters, and chronoscope.

A2.5.3 Use of the Langley Complex Coordinator in Tektite I

The test equipment (Fig. A21) can be programmed to require the subject to respond by matching lights on the display. The response can require one or two hands, one or two feet, or any combination of hands and feet, either concurrently or sequentially. In Tektite I all four banks of stimulus lights were used, requiring concurrent alignment of the response lights. During any one trial it was necessary to hold the controls steady while hunting for any remaining responses to complete the set of four. The display panel contains 45 lights, 40 of which are used to match pairs. The other five lights are used to provide information to the operator or to add complexity to the response required. There are four banks of colored lights, each of which presents a position stimulus, and parallel to them are four banks of matching color lights activated by the responding operator.

In addition there is an interval timer that can be set for varying periods up to 15 seconds. The timer automatically returns to zero when each trial is successfully concluded. A red light goes on if this interval is exceeded by the operator. The operator can reduce or increase this time period as desired, thus pacing his efforts. The test program is preset on a revolving drum attached to an electromechanical timer (Fig. A22). The operator received immediate knowledge of this performance on each trial and for the complete cycle of 50 trials.

Fig. A21 - Operator in position to use the Langley Research Center complex coordinator

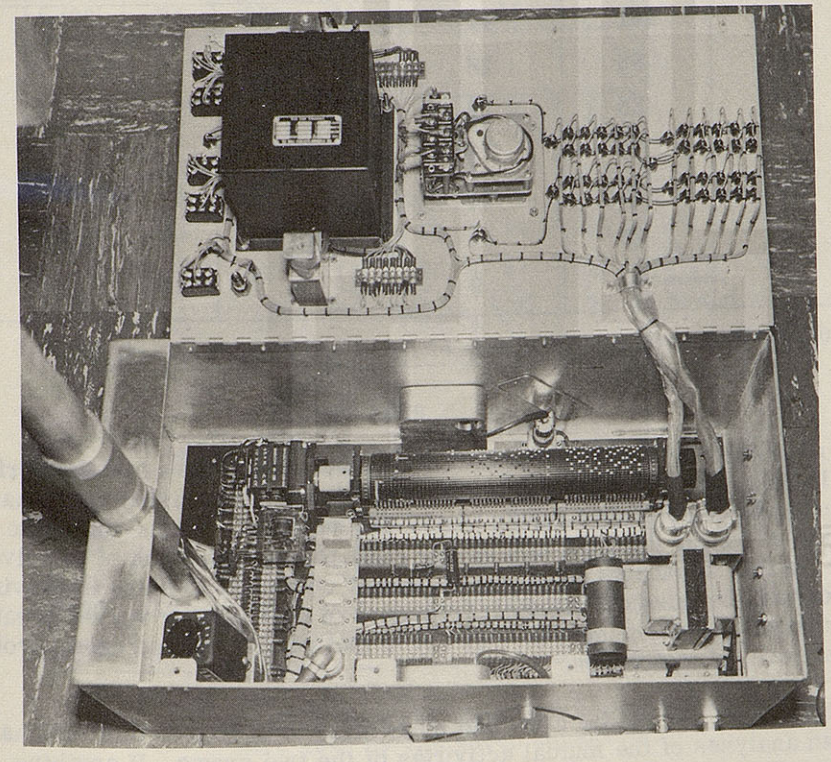


Fig. A22 - Programming drum and electronic features of the LRC complex coordinator

A2.5.4 Preliminary Results

It was planned that each of the four aquanauts in the Tektite I habitat and the three backup aquanauts at the base camp would perform the series of psychomotor tests daily. The backup aquanauts acted as a control group for the underwater divers. Due to the ear infection of the aquanauts and other overriding factors the tests were not performed as frequently as scheduled. The actual days during which the Langley complex coordinator was used is shown in Fig. A23. The dates on which the underwater crew, composed of Clifton, Mahnken, Van Derwalker, and Waller (not in that order), performed on the psychomotor tester, during the actual dive, are shown in Fig. A24.

The frequency of performance and number of cycles attempted are shown in Table A9 for each aquanaut. A cycle consists of a series of 50 trials and represents one rotation of the programmer drum. A trial is the solution to one problem, i.e., the matching of one group of four lights concurrently. For analysis purposes the data for the trials and cycles were gathered on the data cards shown in Fig. A25. The testing series was introduced during the orientation and training phases starting on December 11, 1968, and continuing up to a few days prior to the 60-day dive.

Table A9
Total Use of the Langley Research Center Complex Coordinator

Aquanaut		Total Number of Cycles*	Training Cycles	Cycles During the Test		Days Used During the Test
Diving	Backup			Diving Aquanauts	Backup Aquanauts	
VII		151	17	134		14
	V	50	28		22	7
	VI	38	29		9	5
III		73	15	58		7
	IV	49	20		29	4
I		72	18	54		10
II		15	9	6		5

*A cycle is 50 trials of matching one group of four lights concurrently.

In almost all cases the aquanauts achieved a plateau (asymptote) on the performance curves shown in Fig. A26. In every case, performance on the complex coordinator by the surface aquanauts deteriorated during the 60-day mission. In one case, one of these backup aquanauts no longer used the test device after the second week of the dive. On the other hand the four aquanauts living in the habitat continued to show improving performance throughout the mission, although frequency of self-testing varied greatly among them. This improving performance, in contrast to that of the surface crew, probably indicates a higher level of motivation.

The possibility that the habitat crew had more time to devote to this task has been discounted by an analyses of the mutual activities of the two crews. If anything, the habitat crew had less time available for this peripheral task than the surface group. The undersea crew continually expressed their willingness and desire to use the complex

DECEMBER 1968

S	M	T	W	T	F	S
1	2	3	4	5	6	7
8	9	10	(11)	(12)	(13)	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30	31				

At General Electric

JANUARY 1969

S	M	T	W	T	F	S
			1	2	3	4
5	(6)	(7)	(8)	(9)	(10)	11
12	13	14	15	16	17	18
19	20	21	22	23	24	25
26	27	28	29	30	31	

At the University of Pennsylvania Hospital

FEBRUARY 1969

S	M	T	W	T	F	S
						1
2	3	4	5	(6)	(7)	8
9	10	(11)	(12)	13	14	15
16	(17)	(18)	(19)	20	(21)	22
(23)	24	(25)	(26)	27	28	

MARCH 1969

S	M	T	W	T	F	S
						1
(2)	3	4	(5)	(6)	7	(8)
9	10	(11)	(12)	13	14	15
16	(17)	18	(19)	20	21	22
23	(24)	(25)	(26)	(27)	(28)	29
30	(31)					

APRIL 1969

S	M	T	W	T	F	S
		1	(2)	(3)	(4)	(5)
6	7	8	(9)	10	(11)	(12)
(13)	(14)	15	16	17	18	19
20	21	22	23	24	25	26
27	28	29	30			

At St. John Island

Fig. A23 - Days the complex coordinator was used by the seven aquanauts

FEBRUARY 1969

S	M	T	W	T	F	S
						1
2	3	4	5	6	7	8
9	10	11	12	13	14	(15)
16	17	18	(19)	20	21	22
(23)	(24)	25	(26)	27	28	

(15) — Day submerged; no use of test on this day

MARCH 1969

S	M	T	W	T	F	S
						1
(2)	3	4	(5)	(6)	7	8
9	10	11	(12)	13	14	15
16	17	18	(19)	20	21	22
23	24	(25)	(26)	(27)	(28)	29
30	(31)					

APRIL 1969

S	M	T	W	T	F	S
		1	(2)	(3)	4	5
6	7	8	(9)	10	(11)	(12)
13	(14)	15	16	17	18	19
20	21	22	23	24	25	26
27	28	29	30			

Day surfaced — 15

Fig. A24 - Days the complex coordinator was used by the four aquanauts while underwater in the Tektite I habitat

coordinator. In fact two of the aquanauts competed with each other by trying to beat the score of the other diver. At the end of the 60-day dive their scores were comparable and still improving at a slow rate.

A2.5.5 Conclusions

It can be concluded that the experience in the Tektite I habitat did not affect performance on a complex psychomotor task as measured by the Langley complex coordinator. These tests were made prior to and after swimming excursions. Therefore, it may be assumed that the scores were not directly affected by fatigue caused by the submerged swimming. In fact several of the aquanauts felt that the complex coordinator presented an interesting and challenging activity that tended to provide relaxation and a unique change in activity.

NAME _____

DATE	HOUR	TEST	IT[2]	RL[3]	TEST TIME[4]
			.		: .
			.		: .
			.		: .
			.		: .
			.		: .
			.		: .
			.		: .
			.		: .
			.		: .
			.		: .
			.		: .
			.		: .
			.		: .
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Fig. A25 - Data analysis card used with the complex coordinator. The columns identified in this figure by the superscripts are (1) sequence of cycle, (2) interval timer setting in seconds, (3) red light frequency count (number of times the interval time was exceeded), and (4) total time to do 50 trials (one cycle) in minutes and seconds.

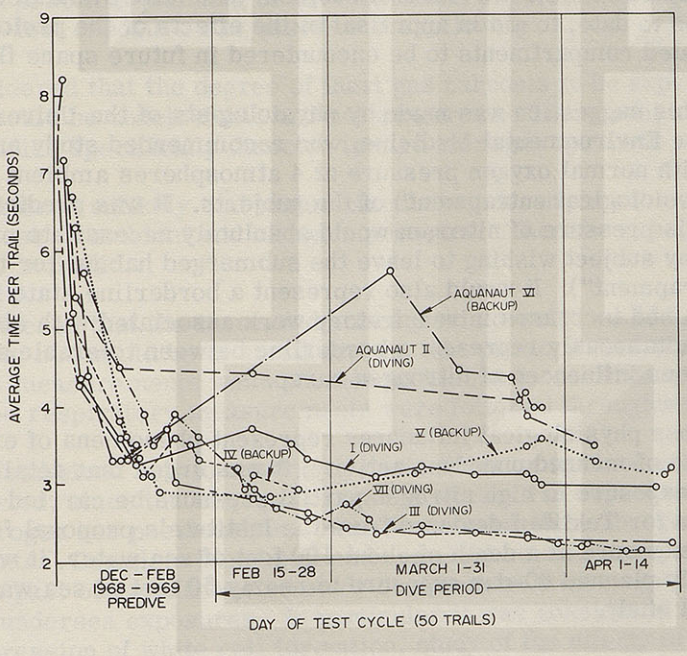

Fig. A26 - Performance of the four diving aquanauts and the three backup aquanauts

This is a preliminary analysis prepared for this overall Tektite I report. Additional analyses are underway to compare periods of activity and test scores with other observations, time spent in the water, mood adjective checklist data, and other performance measures for a more detailed evaluation. More detailed analysis comparing scores across cycles, preceding tasks, and times of the day, is planned.

The Langley complex coordinator worked well throughout the mission. Data from additional testing with the six-man crew of the *Benjamin Franklin* are still being analyzed. It is also planned to use this psychomotor tester in the long-term test and evaluation of integrated life support equipment in which four subjects will be confined in a chamber for 90 days.

A3 BIOMEDICAL SCIENCES

A3.1 Introduction

C. J. Lambertsen, Institute for Environmental Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, and S. Kronheim, Physiology Branch, Office of Naval Research, Washington, D.C.

Project Tektite I, while ultimately applied to marine sciences programs, had its origins in two interrelated fields of human bioscience. U.S. Navy psychologists proposed to the National Aeronautics and Space Administration that open-water, undersea habitats should provide more realistic restriction of subjects than have simulated laboratory confinement studies to date, to aid in appraisal of the effects of the prolonged, enforced restriction in isolated compartments to be encountered in future space flights.

Extension of this suggestion was made by physiologists of the University of Pennsylvania's Institute for Environmental Medicine, who recommended study and use of exposure to nitrogen with normal oxygen pressure at 4 atmospheres ambient pressure to provide a true "physiological entrapment" of the subjects. It was predicted that saturation exposure to this pressure of nitrogen would absolutely necessitate programmed decompression for any subject wishing to leave the submerged habitat for the surface (hence "physiological entrapment"). It would also represent a borderline state for study of the effects of the sustained increases in respiratory work associated with elevated gas density and would simultaneously represent a borderline between tolerable and disadvantageous central nervous influences of nitrongen narcosis.

Since all of these physiological influences represent phenomena of extreme importance to the advance of manned undersea activity, it was urged that detailed biomedical study of sustained exposure to high nitrogen partial pressure be carried out. While the overall project plan for Tektite I deviated from the Institute's proposal for the nitrogen-oxygen saturation exposure at a depth of about 100 feet of sea water, it was clear that even the secondarily planned 60-day exposure to nearly 50 feet of sea water required detailed biomedical study.

A3.1.1 Purposes

The purposes of the biomedical program for Tektite I included:

1. Stimulation of interest in detailed physiological study of nitrogen as a diluent for oxygen in prolonged, shallow-water diving.

2. Performance of the specific medical, physiological, hematological, and microbiological examinations and measurements required to (a) assure qualification of the

subjects to serve as aquanauts, (b) obtain the detailed base-line measures required to assess the importance of any pathological or physiological changes which might develop over the course of the 60-day exposure and (c) obtain clues to possible physiological or pathological alterations which might be encountered in future exposures of greater depth or greater duration.

It was expected from the beginning that, regardless of whether a particular manned open sea project such as Tektite I or Sealab were to continue, the full biomedical exploration of nitrogen-oxygen and nitrogen-helium-oxygen atmospheres will remain important to the extension of general undersea activity.

A3.1.2 Selection of the Program

A biomedical experiments/medical safety planning group was recommended and formed to devise the experiment program, to assure safe diving and decompression procedures, and to anticipate potential risks to the health and safety of the aquanauts and support personnel. This group, representing the several major participating organizations, was comprised of: C. J. Lambertsen (Institute for Environmental Medicine, University of Pennsylvania), biomedical/medical safety program coordination; S. Kronheim (Physiology Branch, Office of Naval Research), biomedical experiments program coordination; Cdr. T. N. Markham (Naval Medical Research Laboratory), on-site medical monitoring; Capt. E. L. Beckman (Manned Spacecraft Center, NASA), NASA liaison; and S. Gottlieb (General Electric Company), human engineering liaison.

Conceptual and practical planning of the biomedical experiments/medical safety program for the 50-foot exposure was based on the following:

- It was considered that the degree of inert gas narcosis to be expected at a depth of 50 feet or less would be undetectable and not dangerous. Therefore, no physiological or performance studies specifically directed toward inert gas narcosis were included.

- It was considered unlikely that major circulatory derangements would result from the moderate increase in ambient and nitrogen pressure. Therefore, no extensive circulatory studies were included.

- It was considered that the increased atmospheric density would be the primary physiological stress, but would be of small degree. Respiratory control and detailed pulmonary function measurements were planned as the most sensitive indices of such stress, and selected respiratory measurements were followed throughout the exposure.

- Changes in red blood cell formation or destruction or in blood chemical composition were not expected, since the inspired oxygen pressure was kept near a normal sea-level value and nitrogen has previously not been found to produce changes in formed or chemical constituents. Complete blood cell and chemical studies were nevertheless conducted to provide a part of a multifaceted study of abnormal atmospheres ranging from aerospace to deep undersea exposures. In particular it was considered that, since nitrous oxide induces suppression of white cell formation, study of the effects of chronic exposure to nitrogen on white cell formation was essential.

- Study of microbiological alterations of the habitat interior surfaces, the respired atmosphere, and especially the skin, upper respiratory tract, gastrointestinal tract, and auditory canals of the subjects was considered desirable. This interest stemmed from the conditions of closely confined residence and repeated wetting of the skin and included the potential for exchange of organisms with the surrounding environment.

- Detailed dermatological studies with quantitative microbiological counts were included to assess the reasonable possibility that skin softening, chronic skin wetting, and bacterial or mold infections of the skin might prove a major limiting factor in prolonged submergence.

- Study of sensory functions, including vision, hearing, and vestibular function, was prompted not only by concern for any neurological influences of nitrogen at increased partial pressure but out of concern for subtle influences of bubble formation.

- Study of special decompression requirements was considered essential, since no evaluation of decompression procedure for nitrogen saturation diving at depths greater than 30 feet had previously been carried out.

Responsibilities for execution of studies in each major area were assigned by the biomedical experiment/medical safety group to individual investigators who carried out the detailed planning, supervision, and measurement.

A3.1.3 Facilities and Personnel

Successful conduct of the biomedical experiments/medical safety program depended on several organizations and a large number of dedicated investigators.

General medical and medical specialty examinations depended on the following investigators from the University of Pennsylvania: C. J. Lambertsen, Institute for Environmental Medicine; H. M. Rawnsley and C. Shute, Clinical Research Center; T. W. Clark, Diagnostic Clinic; W. S. Masland, Electroencephalography Unit; A. M. Kligman and R. R. Marples, Department of Dermatology; M. Reivich, Department of Neurology; C. W. Nichols, Department of Ophthalmology; W. K. H. Sundmaker, Department of Otolaryngology; and R. H. Chamberlain, Department of Radiology.

Decompression studies were by the Medical Research Laboratory, U.S. Naval Submarine Medical Center, New London, Connecticut (Cdr. T. N. Markham); National Aeronautics and Space Administration, Manned Spacecraft Center, Houston, Texas (Capt. E. L. Beckman); and J and J Marine Diving Company, Inc., Pasadena, Texas (Peter O. Edel).

On-site medical monitoring was by Cdr. T. N. Markham, U.S. Naval Submarine Medical Center; Lt. P. V. Van Tassel, Bureau of Medicine and Surgery; J. G. Dickson and C. J. Knight, Institute for Environmental Medicine, University of Pennsylvania Medical Center; Cdr. M. E. Bradley and Lt. Cdr. J. Vorosmarti, Deep Submergence Systems Project, San Diego; and Cdr. J. C. Rivera, 10th Naval District, San Juan.

Hematology studies were by C. L. Fischer, Manned Spacecraft Center, NASA, and P. C. Johnson, College of Medicine, Baylor University.

Microbiological studies were by Lt. A. B. Cobet, Naval Biological Laboratory, Oakland.

Respiratory/pulmonary and physiological studies were by investigators from the Institute for Environmental Medicine and Department of Physiology, University of Pennsylvania Medical Center: J. G. Dickson, A. B. DuBois, A. B. Fisher, R. Gelfand, R. W. Hyde, C. J. Knight, and C. J. Lambertsen.

A3.2 General and Special Medical Examinations

A3.2.1 General Objectives, Rationale, and Procedures

C. J. Lambertsen, Institute for Environmental Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, and Cdr. T. N. Markham, Naval Submarine Medical Center, New London, Connecticut

A3.2.1.1 General Objectives

A dominant purpose of Tektite I was to establish the general safety and operational usefulness of prolonged undersea exposure to high nitrogen pressures. To make real determination of physiological or medical risk it was necessary to design and execute exceptionally detailed and comprehensive biomedical studies of both a clinical and fundamental nature. These correlated evaluations of the aquanaut subject group were conducted in full detail prior to acceptance of a subject for the submerged exposure and again during the 2 days immediately following ascent to the surface. During the period of exposure a more limited, but still extensive, appraisal was conducted.

Because of the extent of the special clinical and physiological examinations employed, these preexposure studies were carried out by a combined staff of the University of Pennsylvania's Institute for Environmental Medicine and Clinical Research Center. In all of these special examinations the individual specialists who performed the initial, preexposure evaluation also performed the same special examination for the postexposure period at the diving site. Medical monitoring at the diving site was carried out by a team of physicians representing the U.S. Navy and the University of Pennsylvania.

A3.2.1.2 Preexposure and Postexposure Clinical Studies

The biomedical experiments/medical safety committee considered that, aside from the general clinical appraisal, respiratory and pulmonary measurements, microbiological studies, and hematological monitoring that were part of the overall program, several clinical specialty examinations deserved to be included in the aquanaut assessment. These were: ophthalmological, to involve complete study of ophthalmological and visual status; dermatological, to include detailed quantitative study of skin flora and skin permeability characteristics, as well as development of skin disease; neurological, to include complete neurological examination and clinical electroencephalograms; audiovestibular, to involve quantitative measurement of hearing and determination of vestibular function; and radiological, to include examination of the lungs, skull, and gastrointestinal system.

A3.2.1.3 Status Assessment Examinations During Submergence

Because overall design of the Tektite I project required nearly complete isolation of the aquanauts during submergence, medical status examinations were performed by the aquanauts themselves. They were trained to use simple diagnostic equipment such as the aneroid sphygmomanometer and stethoscope for blood pressure measurement, otoscope, oral probe for a telethermometer, and electrocardiographic leads. A medical questionnaire was devised to permit systematic review and reporting of any positive responses.

A3.2.2 General Medical Examinations

A3.2.2.1 Preexposure Examinations

T. W. Clark, Diagnostic Clinic, University of Pennsylvania

The general medical appraisal examinations prior to exposure were conducted at the Diagnostic Clinic of the University of Pennsylvania hospital. They included: medical

history; physical examination; electrocardiogram, both resting and double Masters exercise recordings; ballistocardiogram; radiological examination of the skull (as part of neurological examination), chest (anterior-posterior and lateral), and upper gastrointestinal tract; laboratory examinations of blood elements (hemoglobin concentration, red cell count, white cell count, differential white cell count, and platelet count); blood chemistry (urea nitrogen, creatinene, protein-bound iodine, cholesterol, and glucose 2-hour postprandial concentration); blood serology; urinalysis; and stool-occult blood.

Results of these examinations, stored as part of the Tektite I program record, indicated that no limiting abnormalities were present in the subject group. As normal precautionary measures each aquanaut was brought to current immunization against smallpox, typhoid fever, tetanus, poliomyelitis, and Hong Kong influenza.

A3.2.2.2 Examinations During Submergence and Immediately Postexposure

Cdr. T. N. Markham, Naval Submarine Medical Center

Initially the medical status was reviewed by the subjects daily, to include a report to the surface medical watch of body weight, oral temperature, blood pressure, pulse frequency, dermatological inspection, and auditory canal, drum, and throat inspection. After the first 2 weeks these reports were made only every 2 days. Once weekly each subject had an electrocardiogram (six leads weekly, 12 leads once per month); these tracings were sent to the surface for interpretation. Throughout the exposure any specific complaints or symptoms were reported and investigated when they arose. Weekly the pulmonary-function information was made available to the medical monitors, as was complete blood-count information from the hematology study. Bacterial culture results were available immediately following incubation and isolation at the site. On decompression, general medical appraisal was made immediately, then followed over the succeeding 2 days by detailed clinical examination.

The general medical monitoring throughout the exposure, together with the postexposure examinations uncovered no limiting abnormalities. The only significant medical conditions arising during the saturation exposure included the following:

- Aquanaut 1 reported paresthesias and weakness of his right hand and wrist during the first week on a day following extensive manual labor with his right arm. During this manual labor his arm had been in an abnormal position. The condition was diagnosed as radial nerve palsy and gradually cleared over the following 10 days. The subject was restricted from diving for 1 day.

- During the saturation phase between March 3 and March 23, 1969, all four subjects developed otitis externa; aquanauts 1 and 4 had bilateral infections with each ear infection separated by 5 to 7 days. Planned prophylactic use of ethanol cleaning and drying of the ear canal was not routinely carried out at the beginning of the exposure. The infections responded poorly to chemotherapy with corticosporin otic drops, possibly because of the presence of the corticosteroid. They responded rapidly to systemic tetracycline and colymycin otic drops. Following the infections each aquanaut instilled a mixture of ethanol and boric acid in each ear after each dive. This procedure appeared to prevent further recurrence.

Table A10 summarizes the average values of general vital signs over the period of submergence.

Table A10
Vital Signs During the Saturation Phase

Aquanaut	Weight (lb)				Temperature (°F)*		Radial Pulse		Blood Pressure				
									Systolic		Diastolic		
	Initial	Final	Mean	Std Dev	Range	Mean	Range	Mean	Range	Mean	Range	Mean
1	155.0	157.0	156.9	1.5	98.0-99.9	98.8	104-76	90	130-106	117	86-64	75
2	161.0	157.0	158.5	1.0	97.3-99.5	98.4	100-72	85	130-110	117	84-50	73
3	185.0	183.0	183.9	1.0	97.8-99.2	98.5	84-58	71	130-102	116	88-68	77
4	158.0	150.0	153.6	1.9	98.2-99.4	98.8	92-72	81	138-105	125	106-68	85

*The thermometers were not calibrated for the 42' depth; the 'above normal' temperatures are due to the increased pressure.

A3.2.3 Auditory-Vestibular Examination

W. K. H. Sundmaker, Department of Otolaryngology,
University of Pennsylvania

A3.2.3.1 General Objectives

The purpose of the complete ear, nose, and throat examination was to detect preexisting abnormalities which would limit performance during or be exacerbated by prolonged submergence. The scope of the special examination was expanded to include audiometric survey and study of vestibular function by neurological and caloric methods.

A3.2.3.2 Results

Full documentation of pre- and postexposure examinations is stored with the Tektite I records. No limiting defects were found on preexposure examination. Postexposure examinations were made between 13 and 20 hours after the end of decompression. No subject showed any sign of vestibular dysfunction, and there were no subjective changes in hearing or other signs of auditory disturbance. For technical reasons a postexposure audiogram could not be done at the test site. Although no permanent threshold shift should have resulted from the exposure, all subjects were advised to have a repeat audiogram within 2 months.

The labyrinthine responses to cold calorization were generally depressed in all subjects as compared to the preexposure tests. This is in agreement with the observation made by others that vestibular nystagmus is enhanced by arousal and diminished by fatigue.

Although all subjects had had external otitis during the early period of submergence in the habitat, this had subsided at the time of the post exposure examination. Only aquanaut 3 had traces of grayish-green pigmented debris in his ear canals, presumably residua of *Pseudomonas* infection.

A3.2.3.3 Conclusion

Within the limits of this examination and in the absence of subjective complaints that would have called for more specialized tests, none of the subjects appeared to have sustained any damage to the vestibular apparatus or conductive auditory system, and no permanent changes in neurosensory auditory function are to be expected.

A3.2.4 Dermatological Examinations

A. M. Kligman and R. R. Marples, Department of
Dermatology, University of Pennsylvania

A3.2.4.1 General Objectives

It was considered probable that repeated and prolonged wetting of the skin could lead to deterioration of cutaneous function, susceptibility to trauma, and increased likelihood of infection. Complete dermatological examination was used as a preexposure base line for determining the nature and degree of any alterations which might be produced by the prolonged submergence. General inspection throughout the exposure was performed by the subjects. The detailed postexposure examinations were conducted within the first morning after completing decompression.

A3.2.4.2 Qualitative Aspects

The four participants had no noteworthy dermatologic disorders prior to immersion. They even lacked signs of athlete's foot, which is common in young men. After surfacing, each was in a state of excellent dermatologic health. Careful search failed to disclose even dandruff or an occasional folliculitis of the beard hairs. Obviously the habitat provided excellent opportunities for prophylactic care of the skin. Cleanliness and especially drying out between dives and at night are deemed to be the crucial factors which enabled the aquanauts to emerge with fewer skin conditions than they would have developed in the base camp for the same period.

Two subjective features should be mentioned. Three of the four subjects thought that there was slower growth of scalp and beard hair. This is inexplicable and may or may not be a reliable observation. Secondly all reported softer nails which were easily torn. The subjects did not have to cut their nails, but this may reflect the work they performed, which probably wore away the free edge of the nail plates. The softer nails doubtless resulted from hydration and not from internal factors which might result in defective nail formation.

Skin biopsy was taken from the volar surface of the forearm before immersion, but repetition of biopsy was eliminated from possibility after decompression.

A3.2.4.3 Preexposure and Postexposure Quantitative Tests

A3.2.4.3.1 Introduction

Scrub samples for corneocyte counting and quantitative bacteriology were taken using the methods of McGinley, Marples, and Plewig* and Williamson and Kligman† from each side of the forehead and each volar forearm. These studies were intended to provide quantitative measures of changes in flora to supplement the qualitative studies comprising the microbiology program. Swab samples from the fourth interspace of each foot were taken by ten full strokes of a Triton X-100 moistened swab which was returned to 1 ml of wash fluid. The samples obtained at the diving site were transported in a vacuum flask containing ice and were plated the same evening in Philadelphia.

A3.2.4.3.2 Corneocytes

On the forearm the geometric mean corneocyte count fell from 168,500 to 139,500. This is of borderline significance. No nucleated cells were seen.

On the forehead the geometric mean count rose from 61,200 to 79,200. This rise is not significant. The level of nucleated corneocytes decreased slightly.

In the toeweb samples the geometric mean count fell markedly from 315,000 to 54,200 ($p < 0.01$). However, the initial samples were taken in Philadelphia, where the subjects had been wearing shoes, and in the submerged habitat this was not the case.

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*K. J. McGinley, R. R. Marples, and G. Plewig, "A Method for Visualizing and Quantitating the Desquamating Portion of the Human Stratum Corneum," J. Invest. Dermatol. 53, 107-111 (1969).

†P. Williamson and A. M. Kligman, "A New Method for the Quantitative Investigation of Cutaneous Bacteria," J. Invest. Dermatol. 45, 498-503 (1965).

A3.2.4.3.3 Bacterial Densities

The scrub samples taken for corneocyte counts were also examined by techniques of quantitative bacteriology.

On the forearm the aerobic bacterial density rose from a geometric mean of 223 to 558. The flora included more aerobic spore formers and fewer cocci in the postimmersion samples. The density of *C. acnes* on the forearm fell from 3560 to 670.

On the forehead the aerobic density rose slightly from 14,300 to 54,600. However, the density of *C. acnes* fell sharply from 42.3×10^6 to 4.07×10^6. This fall is highly significant. Perhaps this is due to the prolonged and motivated use of hexachlorophene antibacterial soap, although the rise in aerobic density does not confirm this hypothesis.

A3.2.4.3.4 Conclusions

The four participants had no noteworthy dermatologic disorders prior to immersion. The postulated occurrence of severe dermatologic disorders was not encountered.

It is probable that the maintenance of normal skin condition was related to the combination of relatively low humidity in the habitat, avoidance of excessive temperature in the habitat, relatively short periods of work in the water as compared with time spent in the gaseous environment, the ready availability of fresh water for washing of skin and clothing, and the use of a bacteriocidal soap for the frequent showers. The major dermatologic failure can be considered the infections which occurred in the skin of the ear canal. These sites were initially allowed to remain wet instead of being dried and had instillations of water-holding glycerine instead of the more rational water-removing and bacteriocidal ethanol.

It should be recognized that, from a dermatologic standpoint, conditions in the habitat were nearly ideal — certainly superior to conditions in the base camp. While no problems developed in the aquanaut-subjects, it can be expected that warmer climate, higher humidity, poorer hygiene, and increased daily duration of diving will lead to dermatological changes including infection and physical breakdown.

A3.2.5 Neurological Examination

M. Reivich, department of Neurology, and W. S. Masland,
Electroencephalography Unit, University of Pennsylvania

Since exposure to increased nitrogen pressure in saturation diving or the use of oxygen at high pressure in bends therapy could induce central nervous system effects, complete neurological examination was performed as part of the base-line selection appraisal of each subject. The objective of these clinical neurological examinations was to detect preexisting neurological abnormalities, whether limiting or not.

The preexposure studies included skull x-rays, electroencephalograms, and detailed neurological examinations. The latter consisted of an assessment of each subject's mental status, station and gait, cranial nerves, cerebellar function, motor function, sensory function, and reflexes and an examination of the extracranial cerebral vessels. No limiting abnormalities were found in the preexposure examination.

Following decompression a second complete neurological examination was carried out within 48 hours by the same neurologist who had performed the preexposure examinations. No changes from the preexposure examination were found.

A3.2.6 Ophthalmological Examinations

C. W. Nichols, Department of Ophthalmology, University of Pennsylvania

A3.2.6.1 General Objectives

The initial examination was devised to assure that all individuals taking part in the Tektite I Project had not had or did not have any significant ocular disease such that a recurrence or exacerbation would threaten their vision. This examination, additionally, provided an extensive base line so that any deviation from the individual's ocular norm could be adequately investigated. The major investigative task of the ophthalmologic program was to determine if any changes in visual function occurred during the saturation dive and to evaluate the eye for structural changes that might occur during diving and subsequent decompression.

A3.2.6.2 Content of the Examination

The initial examination was carried out in the eye clinic of the University of Pennsylvania hospital. The tests used were those which constituted part of the routine workup of all patients as well as certain specialized examinations so chosen that conceivably they could be repeated in a field situation. The requirement to be able to repeat the test at the project site eliminated certain electrophysiological measurements (electroretinogram measurements), which although possibly desirable have not proved of significance in past investigations. An outline of the initial examination follows.

- Ocular history with emphasis on: history of injury to the eye or adnexa; previous visual difficulty, particularly if associated with diving or other hyperbaric exposure; and recurrent infections of cornea, conjunctiva, or lids.

- Visual acuity with and without correction at distance (20 feet) and near (14 feet) and measurement of accommodative ability.

- External examination: notations made of lids, lashes, fissures, conjunctiva, cornea, and lacrimal system.

- Pupillary responses to light and accommodation.

- Evaluation of extraocular muscle balance to include: primary position and versions, measurement of near and distant phorias (tropias if indicated), and vertical and horizontal fusional amplitudes.

- Central visual fields by tangent screen with white and colored test objects.

- Slit-lamp examination of the undilated pupil.*

- Shiotz and/or applanation tonometry*.

- Slit-lamp examination of the dilated pupil.*

- Refraction — cycloplegic (Mydriacyl).

- Photography of disk and macular areas.*

*Could not be done at the project site.

- Fluorescein angiography (arm to eye circulation time) and photography of the macular pattern (to be repeated at project site if indicated).

- Examination of the fundus and ocular media by direct and indirect ophthalmoscopy.

A3.2.6.3 Summary of Significant Findings

In all cases the preexposure findings were typical of those found in young healthy individuals. No ocular abnormalities of any type were noted in the subjects except for a moderate myopic astigmatism correctible by lenses in aquanaut 3 and a higher than normal intraocular tension in aquanaut 2. Neither of these was felt to have significance in terms of the planned saturation diving exposure.

There was no postdecompression alteration in the visual function of any of the subjects as evaluated by the parameters measured. Three alterations were detected, however. In aquanaut 1 marked injection and mild chemosis of the conjunctivae were present. This was attributed to a sensitivity to environmental contaminants in the habitat. This subject found that the condition was exacerbated while changing the baralyme canisters, and presumably chemical dust produced in this activity was a cause of much of his problem. It was in no way incapacitating.

Aquanaut 3 was found to have a decrease in his convergence amplitude. He was accustomed to using stereoscopic equipment in his work before the dive and "crossing his eyes" to obtain a stereoscopic effect. He did not do this during his period in the habitat, and presumably lack of practice accounted for his decreased total convergence amplitude.

During the preexposure examination of aquanaut 2 no abnormalities were noted in his lens by either direct ophthalmoscopy or slit-lamp examination. However, after decompression a small spherical region approximately 0.5 mm in diameter was easily noticed by two observers by means of a direct ophthalmoscope to be present in the lens of the right eye. This was at about 10 o'clock peripherally and did not affect vision. He was referred upon returning home to an ophthalmologist, who reported only a very small peripheral spherical defect in the right eye at about 12:30 seen with great difficulty with the slit lamp. The originally detected spherical area was at that time not present. Followup examination by the original observers 6 months after exposure also showed only the unrelated minute defect seen at 12:30 by the consultant ophthalmologist. This was extremely small and difficult to see; it was not in the area of the defect noted immediately postdecompression. From the structure and normal progression of lens changes, it can be surmised that the defect observed on return to the surface was related to decompression and was most probably a gas bubble which went on to complete resolution. The unfortunate inability to follow up this observation on a continuous, day-by-day basis was due to scattering of personnel from the diving site following completion of the operation.

A3.3 Hematology

A3.3.1 General Objectives

C. L. Fischer, Preventive Medicine Division,
NASA Manned Spacecraft Center, Houston, Texas

The general objectives of the hematology program were to describe the hematologic, immunologic, and biochemical implications incident to 60 days of continuous submersion, exposure to a nitrogen/oxygen atmosphere at increased pressure, and confinement in a semiclosed environment and the hazards of prolonged saturation diving. Specifically the studies were designed to provide the following:

- Documentation of participating crew members' physical qualifications, related to the dive, and detection of problems which would require remedial or preventive action.

- Information relative to the etiology, time course and extent of any alterations in red cell mass and/or leukocyte function.

- Information referable to the humoral and cellular components of immunity from crew members exposed to the rigors of long-term saturation diving.

- Data referable to any alterations in fluid and electrolyte balance and musculoskeletal metabolism as reflected by selected biochemical constituents of blood.

- Information about the endocrine system required to objectively quantitate the "physiological costs" or "stresses" incurred by long-term saturation diving.

A3.3.2 Hematology and Radioisotope Studies

P. C. Johnson, Division of Nuclear Medicine,
Baylor University College of Medicine, Houston, Texas

A3.3.2.1 Objectives, Measurements, and Methods

The specific objectives of the radioisotope studies were to study the time course, extent, and etiology of any alterations in circulating red cell mass, red cell survival, and plasma volume incurred by the Tektite I environment.

The following measurements were made (with the methods in parentheses): hematocrit (micromethod), hemoglobin (cyanomethemaglobin), red cell indices (calculation), reticulocyte count (wet and dry methods), white cell count (Coulter counter Model F), differential and morphology (routine methods), photomicroscopy as required (Carl Zeiss Ultraphot), platelet counts (phase microscopy), red cell mass (^{51}Cr in vitro tag), red cell survival (^{51}Cr half-life; glycine ^{14}C in vivo tag), and plasma volume (^{125}I-HSA).

A3.3.2.2 Results

The results are given in Tables A11, A12, and A13.

A3.3.2.3 Discussion

A3.3.2.3.1 Hematology Studies

The routine hematologic parameters (Table A11) demonstrated an unexpected stability, with very few significant trends or isolated findings. An initial intradive decrease in the hematocrit values of John Van Derwalker and increases to levels above predive values for a couple of the divers was seen; however, the postdive hematocrits were slightly below predive norms. The significance of these intradive trends is tenuous; however, the postdive decrease in hemoglobin and hematocrit is related to increases in plasma volume rather than decreases in red cell mass. An asymptomatic, transient eosinophilia occurred in Richard Waller during the dive, the significance of which is not obvious at this time.

Table A11
Hematology Results

Date Relative to D (Dive)	Hemoglobin (g/100 ml)	Hematocrit (%)	Total Count	White Blood Cells										Red Blood Cells	Reticulocytes	Platelet Count	Mean Corpuscular Hemoglobin	Mean Corpuscular Volume	Mean Cell Hemoglobin Content
				Neutrophils		Lymphocytes		Monocytes		Eosinophils		Basophils		Others							
				Pct	Count	Pct	Count	Pct	Count	Pct	Count	Pct	Count	Pct	Count						
Clifton (Diver)																					
D − 30	14.8	43	6380	54	3445	37	2361	5	319	4	255	0	0	0	0	4.75	0.6	165,000	31	91	34
D − 11	14.3	43	5060	59	2985	29	1467	7	354	4	202	1	51	0	0	ND	0.6	247,000	ND	ND	33
D − 5	13.3	41	3520	62	2182	35	1232	1	35	2	70	0	0	0	0	ND	ND	ND	ND	ND	32
D + 14	14.7	44	5500	54	2970	33	1815	6	330	6	330	1	55	0	0	ND	0.3	180,000	ND	ND	34
D + 21	16.2	47	5500	52	2860	40	2200	3	165	4	220	1	55	0	0	ND	ND	190,000	ND	ND	36
D + 28										Clotted											
D + 35	14.8	45	7150	50	3575	41	2932	3	215	6	429	0	0	0	0	ND	ND	120,000	ND	ND	34
D + 42	14.2	43	7510	43	3229	48	3605	4	300	5	376	0	0	0	0	ND	0.3	230,000	ND	ND	33
D + 49	14.8	45	6160	53	3265	35	2156	8	493	4	246	0	0	0	0	ND	0.2	230,000	ND	ND	33
D + 56	14.7	46	6820	58	3956	35	2387	4	273	3	205	0	0	0	0	ND	0.6	250,000	ND	ND	32
D + 60 (bottom)	13.1	40	10000	52	5200	43	4300	4	400	1	100	0	0	0	0	ND	ND	153,000	ND	ND	33
D + 60 (surface)	13.3	41	7260	56	4066	38	2759	4	290	2	145	0	0	0	0	ND	0.2	226,000	ND	ND	33
Mahnken (Diver)																					
D − 30	14.8	42	5720	52	2974	38	2174	2	144	6	343	1	57	1	57	4.78	0.8	188,000	31	88	35
D − 11	15.2	45	5780	45	2601	48	2774	3	173	2	116	1	58	1	58	ND	0.7	151,000	ND	ND	34
D − 5	14.4	42	3850	64	2464	32	1232	0	0	3	116	1	39	0	0	ND	ND	ND	ND	ND	34
D + 14	15.8	46	7480	57	4264	26	1945	5	374	9	673	1	75	2	150	ND	0.7	166,000	ND	ND	34
D + 21	15.0	45	6160	40	2464	49	3018	2	123	6	370	1	62	2	123	ND	ND	185,000	ND	ND	35
D + 28	15.1	46	5060	46	2328	48	2439	0	0	6	304	0	0	0	0	ND	ND	175,000	ND	ND	33
D + 35	15.2	46	6050	57	3449	37	2239	2	121	4	242	0	0	0	0	ND	ND	150,000	ND	ND	35
D + 42	15.0	45	6600	43	2838	49	3234	4	264	3	198	0	0	1	66	ND	0.3	150,000	ND	ND	33
D + 49	15.5	44	6600	44	2904	49	3234	3	198	4	264	0	0	0	0	ND	0.1	185,000	ND	ND	35
D + 56	15.1	45	5170	49	2533	40	2068	7	362	3	155	1	52	0	0	ND	0.3	200,000	ND	ND	33
D + 60 (bottom)	14.0	42.5	7920	64	5069	31	2455	3	238	1	79	1	79	0	0	ND	ND	140,000	ND	ND	33
D + 60 (surface)	13.8	43.0	7150	54	3861	45	3218	1	72	3	215	1	72	0	0	ND	0.2	156,000	ND	ND	32
Van Derwalker (Diver)																					
D − 30	15.8	43.0	7260	53	3848	39	2831	4	290	3	218	1	73	0	0	4.93	0.7	235,000	32	87	37
D − 11	14.4	42.0	3780	37	1399	51	1928	7	265	3	113	1	38	1	38	ND	0.8	194,000	ND	ND	34
D − 5	14.0	42.0	3190	47	1499	49	1563	1	32	2	64	1	32	0	0	ND	ND	ND	ND	ND	33
D + 14	12.2	35.0	3080	45	1386	42	1294	6	185	6	185	1	31	0	0	ND	0.0	*13,000	ND	ND	35
D + 21	15.2	45.0	5500	54	2970	42	2310	2	110	2	110	0	0	0	0	ND	ND	250,000	ND	ND	34
D + 28	15.8	47.0	6050	44	2662	52	3146	2	121	2	121	0	0	0	0	ND	ND	200,000	ND	ND	34
D + 35	15.4	46.0	8140	48	3907	48	3907	2	163	2	163	0	0	0	0	ND	ND	149,000	ND	ND	33
D + 42	15.5	46.0	5830	35	2041	55	3207	4	233	4	233	1	58	1	58	ND	0.2	200,000	ND	ND	34
D + 49	15.6	46.0	4940	38	1881	51	2525	10	495	2	99	0	0	0	0	ND	0.1	225,000	ND	ND	34
D + 56	15.2	45.0	4950	41	2030	50	2475	6	297	3	149	0	0	0	0	ND	0.1	150,000	ND	ND	34
D + 60 (bottom)	14.3	41.0	7590	70	5313	28	2125	1	76	1	76	0	0	0	0	ND	ND	120,000	ND	ND	35
D + 60 (surface)	14.3	42.5	5280	62	3274	32	1690	4	211	2	106	0	0	0	0	ND	0.1	196,000	ND	ND	34

(Table Continues)

Table A11 (Continued)

Date Relative to D (Dive)	Hemoglobin (g/100 ml)	Hematocrit (%)	Total Count	White Blood Cells												Red Blood Cells	Reticulocytes	Platelet Count	Mean Corpuscular Hemoglobin	Mean Corpuscular Volume	Mean Cell Hemoglobin Content
				Neutrophils		Lymphocytes		Monocytes		Eosinophils		Basophils		Others							
				Pct	Count	Pct	Count	Pct	Count	Pct	Count	Pct	Count	Pct	Count						
Waller (Diver)																					
D − 30	15.7	45.0	6930	44	3049	46	3188	4	277	5	347	0	0	1	69	5.04	0.6	158,000	31	89	35
D − 11	15.4	46.5	6380	61	3892	33	2105	4	255	1	64	0	0	1	64	ND	0.6	175,000	ND	ND	33
D − 5	15.2	45.5	5940	41	2435	48	2851	3	178	7	416	1	59	0	0	5.35	0.7	123,000	28.4	85.0	33
D + 14	15.7	46.0	8030	47	3774	40	3212	3	241	10	803	0	0	0	0	ND	0.9	168,000	ND	ND	34
D + 21	15.2	45.0	6510	42	2734	44	2864	4	260	8	521	0	0	2	130	ND	ND	260,000	ND	ND	33
D + 28	15.2	46.0	6930	53	3673	39	2703	1	69	7	485	0	0	0	0	ND	ND	138,000	ND	ND	33
D + 35	15.3	46.0	5720	47	2688	36	2059	3	172	13	744	1	57	0	0	ND	ND	208,000	ND	ND	33
D + 42	15.2	45.0	7370	37	2727	51	3759	8	221	8	590	1	74	0	0	ND	0.6	150,000	ND	ND	34
D + 49	15.6	46.0	6270	41	2571	42	2633	6	376	9	564	1	63	1	63	ND	0.1	200,000	ND	ND	34
D + 56	15.2	46.0	5500	38	2090	45	2475	8	440	8	440	1	55	0	0	ND	0.9	188,000	ND	ND	33
D + 60 (bottom)	14.2	43.0	8300	39	3237	52	4316	4	332	5	415	0	0	0	0	ND	ND	132,000	ND	ND	33
D + 60 (surface)	13.8	42.5	7590	35	2657	54	4099	3	228	8	607	0	0	0	0	ND	ND	200,000	ND	ND	32
Davis (Backup)																					
D − 30	15.2	43.0	4510	36	1624	51	2300	7	316	5	226	1	45	0	0	4.92	1.4	178,000	31	37	35
D − 11	15.9	42.5	3575	38	1359	51	1823	3	107	5	179	2	72	1	36	ND	1.0	235,000	ND	ND	37
D − 5	15.4	45.0	2640	51	1346	42	1109	1	26	5	132	1	26	0	0	ND	ND	ND	ND	ND	34
D + 14	16.0	44.0	6430	54	3472	35	2251	8	514	3	193	0	0	0	0	ND	1.1	306,000	ND	ND	36
D + 21	15.8	44.0	4730	47	2223	44	2081	5	237	1	47	0	0	3	142	ND	ND	205,000	ND	ND	36
D + 28	15.0	45.0	8580	77	6607	13	1115	2	172	7	601	0	0	1	86	ND	ND	167,000	ND	ND	33
D + 35	14.8	42.0	4840	56	2710	38	1839	1	48	4	194	1	48	0	0	ND	ND	191,000	ND	ND	35
D + 42	15.1	45.0	4620	46	2125	26	2125	4	185	3	39	1	46	0	0	ND	0.4	190,000	ND	ND	34
D + 49	14.7	44.0	6270	47	2947	39	2445	7	439	5	314	2	125	0	0	ND	0.3	230,000	ND	ND	33
D − 56	15.3	44.0	5060	61	3087	28	1417	8	405	2	101	1	51	0	0	ND	0.6	178,000	ND	ND	35
D + 60 (bottom)	13.5	42.5	3575	48	1716	40	1430	4	143	5	179	3	107	0	0	ND	ND	158,000	ND	ND	32
D + 60 (surface)	13.8	40.0	3740	51	1907	34	1272	7	262	6	224	2	75	0	0	ND	0.0	201,000	ND	ND	35
Koblick (Backup)																					
D − 30	15.2	43	6270	40	2508	52	3260	6	376	1	63	1	63	0	0	4.82	0.9	205,000	32	69	35
D − 11	14.9	46	8360	78	6521	16	1338	31	251	1	84	0	0	2	167	ND	0.9	194,000	ND	ND	32
D − 5	14.4	43	5500	65	3575	32	1760	0	0	3	165	0	0	0	0	ND	ND	ND	ND	ND	34
D + 14	15.8	45	7700	52	4004	40	3080	6	462	2	154	0	0	0	0	ND	0.3	179,000	ND	ND	35
D + 21	15.2	44	6820	57	3887	36	2455	4	273	2	136	0	0	1	68	ND	ND	160,000	ND	ND	35
D + 28	14.3	43	5170	59	3050	39	2016	1	52	1	52	0	0	0	0	ND	ND	192,000	ND	ND	33
D + 35	14.3	43	6160	58	3573	36	2218	4	246	2	123	0	0	0	0	ND	ND	167,000	ND	ND	33
D + 42	15.7	46	7700	56	4312	40	3080	2	154	2	154	0	0	0	0	ND	0.2	236,000	ND	ND	34
D + 49	14.4	42	6160	57	3511	36	2218	2	123	4	246	0	0	1	62	ND	0.2	217,000	ND	ND	35
D + 56	14.6	43	6160	61	3758	31	1910	6	370	2	123	0	0	0	0	ND	0.2	200,000	ND	ND	34
D + 60 (bottom)	13.3	41	6710	47	3154	45	3020	4	268	3	201	1	67	0	0	ND	0.3	188,000	ND	ND	32
D + 60 (surface)	13.2	40	7260	50	3630	46	3340	2	145	2	145	0	0	0	0	ND	0.0	237,000	ND	ND	33

(Table Continues)

Table A11 (Continued)

Date Relative to D (Dive)	Hemoglobin (g/100 ml)	Hematocrit (%)	Total Count	White Blood Cells										Red Blood Cells	Reticulocytes	Platelet Count	Mean Corpuscular Hemoglobin	Mean Corpuscular Volume	Mean Cell Hemoglobin Content
				Neutrophils		Lymphocytes		Monocytes		Eosinophils		Basophils		Others							
				Pct	Count	Pct	Count	Pct	Count	Pct	Count	Pct	Count	Pct	Count						
Phillips (Backup)																					
D − 30	15.3	44.0	8580	61	5234	35	3003	2	172	2	172	0	0	0	0	4.92	1.2	213,000	31	89	38
D − 11	15.4	44.0	6160	63	3881	30	1848	2	123	2	123	0	0	2	123	ND	0.8	224,000	ND	ND	35
D − 5	14.9	43.0	3960	60	2376	33	1307	0	0	6	238	1	40	0	0	ND	ND	ND	ND	ND	35
D + 14	15.9	46.0	7480	60	4488	31	2319	5	374	3	224	1	75	0	0	ND	0.8	1,300,000	ND	ND	35
D + 21	14.8	43.0	5830	50	2915	37	2157	6	350	3	175	2	117	2	117	ND	ND	350,000	ND	ND	34
D + 28	15.0	45.0	8250	64	5280	30	2475	1	83	5	413	0	0	0	0	ND	ND	240,000	ND	ND	33
D + 35	14.5	42.0	7480	51	3815	42	3142	3	224	3	224	1	75	0	0	ND	ND	177,000	ND	ND	35
D + 42	14.5	43.0	5790	68	5161	24	1822	3	228	4	304	0	0	1	76	ND	0.4	320,000	ND	ND	33
D + 49	15.0	45.0	7370	68	5012	25	1843	6	442	1	74	0	0	0	0	ND	0.1	300,000	ND	ND	33
D + 56	16.0	47.0	7480	70	5236	24	1795	2	150	4	299	0	0	0	0	ND	0.8	230,000	ND	ND	34
D + 60 (bottom)	14.3	43.0	5500	65	3575	24	1320	5	275	6	330	0	0	0	0	ND	0.2	222,000	ND	ND	33
D + 60 (surface)	14.3	41.0	5600	67	3752	24	1344	7	392	1	56	1	56	0	0	ND	0.1	254,000	ND	ND	35

Table A12
Radioisotope Results

Date Relative to Dive (D)	Red Cell Mass (ml)	Red Cell Mass Increment*		Plasma Volume (ml)	Plasma Volume Increment*		Blood Volume (ml)	Blood Volume Increment*		Total Body Hematocrit (%)	Peripheral Hematocrit (%)	Ratio of Total Body Hematocrit to Peripheral Hematocrit
		Ml	Pct		ml	Pct		ml	Pct			
Clifton (Diver)												
D − 30	2040	—	—	3055	—	—	5095	—	—	40	44	0.90
D − 11	1921	− 119	6	3322	+ 267	9	5243	+ 148	3	37	40	0.92
D + 60 (bottom)	2131	+ 210	11	3575	+ 253	8	5706	+ 463	9	37	44	0.84
D + 60 (surface)	2180	+ 259	13	3370	+ 58	2	5550	+ 307	6	39	42	0.93
Mahnken (Diver)												
D − 30	2469	—	—	3371	—	—	5840	—	—	42	44	0.95
D − 11	2468	− 1	< 1	3587	+ 216	6	6050	+ 210	4	41	42	0.98
D + 60 (bottom)	2377	− 91	4	3624	+ 37	1	6001	− 49	1	40	43	0.93
D + 60 (surface)	2320	− 148	6	3619	+ 32	1	5939	− 111	2	39	44	0.89
Van Derwalker (Diver)												
D − 30	1975	—	—	2689	—	—	4664	—	—	42	45	0.93
D − 11	1992	+ 17	< 1	3079	+ 390	15	5068	+ 404	7	39	42	0.93
D + 60 (bottom)	1881	− 111	6	—†	—	—	—	—	—	—	42	—
D + 60 (surface)	1886	− 106	5	3752	+ 673	22	5638	+ 570	11	33	43	0.77
Waller (Diver)												
D − 30	2109	—	—	2688	—	—	4797	—	—	44	46	0.96
D − 11	2186	+ 77	< 1	2725	+ 37	1	4911	+ 114	2	44	47	0.94
D + 60 (bottom)	1998	− 188	9	3328	+ 603	22	5326	+ 415	8	38	43	0.88
D + 60 (surface)	1981	− 205	9	3138	+ 413	15	5119	+ 208	4	39	43	0.91
Davis (Backup)												
D − 11	2236	—	—	3163	—	—	5399	—	—	41	45	0.91
D + 60 (bottom)	2192	− 44	2	3566	+ 403	13	5758	+ 359	7	38	42	0.90
D + 60 (surface)	2210	− 26	1	3736	+ 573	18	5946	+ 547	10	37	42	0.88
Koblick (Backup)												
D − 11	2103	—	—	3014	—	—	5117	—	—	41	42	0.98
D + 60 (bottom)	2096	− 7	< 1	3422	+ 408	4	5518	+ 401	8	38	42	0.90
D + 60 (surface)	2098	− 5	< 1	3493	+ 479	16	5591	+ 474	9	38	42	0.90
Phillips (Backup)												
D − 11	2013	—	—	3025	—	—	5038	—	—	40	43	0.93
D + 60 (bottom)	2077	+ 64	3	3148	+ 123	4	5225	+ 187	4	40	44	0.90
D + 60 (surface)	2108	+ 95	5	3522	+ 497	16	5630	+ 592	12	37	44	0.84

*The D + 60 (bottom and D + 60 (surface) increments are both with reference to the D-11 values.
†Dose infiltrated the plasma volume.

Table A13
Red Cell Survival

Date Relative to Dive (D)	^{14}C — Glycine* (disintegrations/min per mg hemoglobin)						
	Clifton	Mahnken	Van Der-walker	Waller	Davis	Koblick	Phillips
D - 30	5.9	4.9	6.2	5.3	—	5.9	7.8
D - 11	6.4	4.9	7.0	—	6.1	6.4	8.9
D - 5	6.6	5.3	7.0	5.9	6.2	6.5	8.3
D + 14	6.0	5.1	7.1	5.5	6.3	6.4	8.3
D + 21	6.6	4.9	6.9	5.7	6.2	6.4	8.0
D + 28	6.2	4.8	6.6	5.5	6.0	6.0	7.2
D + 35	6.0	5.0	6.0	5.7	6.3	5.9	6.3
D + 42	6.4	5.3	6.1	5.8	5.8	6.2	6.2
D + 49	5.6	4.2	6.0	6.1	5.8	6.7	6.3
D + 56	5.8	4.2	6.0	5.6	5.4	6.3	6.4
D + 60 (bottom)	5.7	4.4	6.0	5.4	6.3	5.6	6.4
D + 60 (surface)	6.1	4.7	5.8	5.7	—	—	—
Half Life — ^{51}Cr in Vitro (days)							
Predive	28	29	29	27	—	—	—
Intradive	28	27	28	27	31	28	28

*Plotted in Fig. A27.

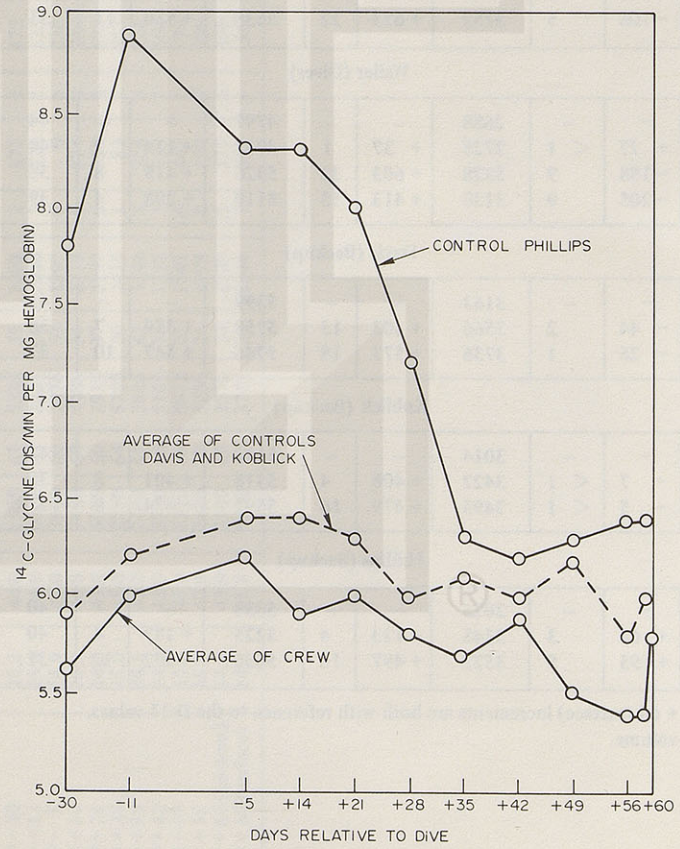

Fig. A27 - Red cell survival as determined by the glycine ^{14}C in vivo tag

A3.3.2.3.2 Radioisotope Studies

Red cell mass, plasma volume, and red cell survival studies were performed on four occasions: twice during the predive control period, on the last day of the dive prior to the decompression, and after surfacing and decompression. The radioisotope methodology used in these studies has been demonstrated sensitive to changes in red cell mass of 2% or more. After consideration of the normal biologic variance and problems encountered by field operations, a variance of 6% is equal to two standard deviations. Plasma volume measurement accuracy is inherently greater; however, the biologic variance of this parameter is considerably larger than that of the red cell mass. This lability is due to the fact that plasma volume responds to certain environmental factors, particularly increased ambient temperatures.

Three of the four divers lost red cell mass (Mahnken, Waller, and Van Derwalker), whereas the fourth individual (Clifton) actually gained red cell mass (Table A12). The control group showed essentially no change in this parameter. Although the red cell mass losses exhibited by three of the divers were at the margin of significance, it must be noted that they are distinctly separable from the control population. The one diver (Clifton) may have had an abnormal transient reduction in his red cell mass at the time of the D - 11 examination; when his D - 30 value is compared to his postdive results, he falls in line with the other divers (+91 ml or 4%). No significant changes in red cell mass were observed as a result of the decompression.

All divers showed alterations in their plasma volumes at some time during the predive, intradive, or postdive intervals. Three of the four exhibited a significant elevation in plasma volume between the D - 30 and D - 11 examinations. The fourth diver (Waller) showed essentially no change during this period. It is probable that the alterations in plasma volumes seen during the predive control period were secondary to the concurrent change in climate experienced by the dive team. During the dive one individual showed essentially no change in plasma volume (Mahnken); whereas, the other divers showed significant increases. It is interesting to note that the diver showing the least loss of red cell mass (Mahnken) was also the man who exhibited the least plasma volume increase. The control group showed similar increases in plasma volume during the predive and intradive periods as did the majority of the dive team.

The ^{51}Cr red cell survival (Table A13) showed no changes throughout the study. The ^{14}C in vivo cohort tag studies showed no differences between the divers and controls, and all values were within the range of normal. It is noteworthy, however, that Mr. Phillips showed (Fig. A27) a very different curve from the other men measured, although his curve is still within the limit of normal.

A3.3.2.4 Conclusions

- No statistically significant changes in red cell mass or red cell survival were detected as a result of the Tektite I dive exposure.

- Plasma volumes increased over the predive and dive intervals in both the control and diver population. This is a probable result of increased ambient temperatures experienced by these personnel after moving to the Tektite I site.

- No significant change in any routine hematologic parameter occurred, with the single exception of a transient eosinophilia in one diver.

A3.3.3 Immunohematology

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A3.3.3.1 Objectives, Measurements, and Methods

Specific objectives of the immunohematology studies were to determine the time course, extent, and etiology of any changes in the humoral and/or cellular immune status of divers exposed to conditions of prolonged saturation diving. This effort will provide the needed data for the safe committal of man to extended dives, particularly those in which prolonged contact with the ocean and ocean floor may be involved.

The following measurements were made (with the methods in parentheses): total serum protein (Goldberg, temperature compensated refractometer), serum electrophoresis (cellulose acetate), immunoglobin quantitation including IgG, IgA, and IgM (single radial immunodiffusion), lymphocyte blastoid transformation (phytohemaglutin stimulation), lymphocyte RNA-DNA synthesis rates (in vitro, ^{14}C and ^{3}H tagging), muramidase (turbidometric method), α2M-globulin (single radial immunodiffusion), transferrin (single radial immunodiffusion), and C'3 — compliment (single radial immunodiffusion).

A3.3.3.2 Results

The results are given in Table A14.

A3.3.3.3 Discussion

No evidence of significant trends is recognized in the immunohematology data, referable to the dive interval. Three of the four divers have consistently abnormal values specifically with respect to γM-globulin (Vanderwalker and Mahnken) and α2M-globulin (Clifton) fractions. The significance of these values is not known.

The significant findings, referable to the cellular immunohematology system, concerns John Van Derwalker, who exhibited a significant reduction in RNA synthesis to PHA stimulation postdive. Since this was not a common finding within the dive group, no overall significance is associated with this event.

A3.3.3.4 Conclusions

The following are the conclusions regarding the divers.

For John Van Derwalker, elevated gamma-globulin levels were recorded during predive intervals, with transient return to normal range on the occasion of the D+60 (bottom) sample. The subsequent D+60 (surface) sampling showed return to the elevated predive levels. This elevated gamma-globulin level was due to a markedly increased γM-globulin level. A transient decrease in lymphocyte RNA synthesis subsequent to PHA stimulation was seen postdive. The significance of this latter finding is unknown.

For Conrad Mahnken, a significantly decreased γM-globulin level was present on all sampling occasions. No significance, referable to the dive, is inferred.

For Richard Waller, no significant abnormalities or changes were noted throughout the examining period.

For Edward Clifton, significant elevations in the α2M-globulin protein fraction was seen throughout the predive and postdive intervals. No protein changes, referable to the dive, were observed.

Table A14
Immunohematology Measurements

Date Relative to D (Dive)	Serum Protein										Muramidase (Lysozyme) (μg/m)	Lymphocyte Response (dis/min per 10^6 viable cells)			
												RNA Synthesis		DNA Synthesis	
	TSP (g-%)	Albumin (g-%)	α2-globulin (g-%)	γ-globulin (g-%)	γG-globulin (mg-%)	γA-globulin (mg-%)	γM-globulin (mg-%)	α2M-globulin (mg-%)	Transferrin	C′3 (mg-%)		Unstimulated	PHA Stimulation	Unstimulated	PHA Stimulation
Clifton (Diver)															
D − 30	6.8	3.7	0.7	1.5	1125	187	185	476	229	77	5.9	1.478	13.386	2.069	16.185
D − 11	7.7	3.6	0.9	2.1	1259	215	206	516	224	64	6.2	4.178	16.047	1.006	20.506
D − 4	7.0	3.6	0.8	1.6	1184	189	182	444	–*	78	5.3	2.300	15.765	1.579	18.807
D + 60 (bottom)	7.1	3.9	0.7	1.5	965	183	147	477	–*	65	4.1	3.000	13.659	0.578	13.028
D + 60 (surface)	7.5	4.0	0.7	1.7	1021	192	168	498	–*	73	5.3	8.066	15.553	3.275	21.568
Mahnken (Diver)															
D − 30	6.7	3.9	0.5	1.3	1027	123	60	335	251	77	7.1	2.865	17.829	1.904	26.036
D − 11	7.7	3.8	0.9	1.9	1197	136	67	335	263	71	10.4	2.305	18.522	2.791	13.048
D − 4	7.2	3.8	0.8	1.6	1080	132	64	282	228	79	6.5	2.500	17.076	2.176	25.958
D + 60 (bottom)	6.7	3.9	0.6	1.2	963	128	61	307	208	62	4.3	5.134	15.914	0.623	11.966
D + 60 (surface)	7.0	4.0	0.6	1.3	997	135	64	328	209	72	4.5	3.017	22.023	4.740	12.968
Van Derwalker (Diver)															
D − 30	7.8	3.6	0.9	2.3	1653	324	364	327	201	74	11.9	2.130	14.873	1.710	11.813
D − 11	7.7	3.7	0.6	2.3	1661	309	368	296	191	62	8.5	0.951	20.011	3.844	23.293
D − 4	7.2	3.7	0.5	2.0	1563	310	328	284	192	62	6.5	1.570	18.695	3.650	25.432
D + 60 (bottom)	7.5	4.1	0.5	1.8	1555	320	336	319	191	51	6.0	4.072	12.083	0.477	13.158
D + 60 (surface)	8.3	4.3	0.6	2.2	1669	366	336	331	207	62	7.4	0.984	12.144	1.631	13.543
Waller (Diver)															
D − 30	7.1	3.8	0.6	1.5	949	132	73	368	261	69	3.8	2.139	13.977	4.875	25.125
D − 11	8.2	4.0	0.9	2.0	1195	153	95	370	285	76	8.2	4.701	14.034	0.628	16.242
D − 4	7.3	3.8	0.7	1.6	979	131	78	315	255	65	5.1	ND	ND	ND	ND
D + 60 (bottom)	7.0	3.9	0.7	1.3	912	135	75	315	244	69	3.0	2.919	19.885	1.937	14.233
D + 60 (surface)	7.3	3.9	0.7	1.5	939	139	75	372	265	78	3.7	4.622	10.416	3.341	11.703
Davis (Backup)															
D − 30	6.6	3.8	0.7	1.2	931	105	182	359	247	81	7.8	3.800	27.325	4.619	16.513
D − 11	7.5	3.8	0.7	1.7	1280	125	260	449	303	101	8.2	2.931	20.560	2.589	10.714
D − 4	7.0	4.2	0.5	1.4	995	107	177	333	245	83	7.0	3.404	21.500	3.599	20.876
D + 60 (bottom)	6.3	3.5	0.6	1.1	848	93	154	320	195	73	5.5	4.284	20.434	0.922	22.602

(Table Continues)

Table A14 (Continued)

Date Relative to D (Dive)	Serum Proteins										Murami dase (Lysozyme) (μg/m)	Lymphocyte Response (dis/min per 106 viable cells)			
												RNA Synthesis		DNA Synthesis	
	TSP (g-%)	Albumin (g-%)	α2-globulin (g-%)	γ-globulin (g-%)	γG-globulin (mg-%)	γA-globulin (mg-%)	γM-globulin (mg-%)	α2M-globulin (mg-%)	Trans ferrin	C′3 (mg-%)		Unstimulated	PHA Stimulation	Unstimulated	PHA Stimulation
Koblick (Backup)															
D − 30	6.8	3.6	0.6	1.5	1035	264	118	301	223	59	8.0	5.359	25.477	4.427	15.538
D − 11	8.0	4.0	0.8	1.9	1259	297	146	312	267	49	15.1	4.255	27.326	1.089	12.570
D − 4	7.9	4.2	0.6	1.8	1171	256	134	284	259	47	7.2	4.952	24.870	3.259	14.202
D + 60 (bottom)	7.2	4.0	0.5	1.5	1048	273	122	293	232	49	7.4	5.206	16.725	2.489	12.290
Phillips (Backup)															
D − 30	7.6	3.7	0.7	2.2	1472	314	200	263	181	65	10.2	2.208	15.219	5.261	18.806
D − 11	8.3	4.0	0.7	2.5	1781	378	260	311	216	65	9.2	4.322	16.181	2.404	17.520
D − 4	7.8	4.0	0.6	2.1	1541	326	182	284	207	69	ND	3.764	16.787	2.956	16.392
D + 60 (bottom)	7.0	4.0	0.5	1.5	1131	282	180	257	195	53	5.8	4.820	15.430	0.379	20.773
Normal Ranges of Values† (±2 standard deviations for plasma proteins and 90th percentile for lymphocyte response)															
Lower value	6.5	3.3	0.5	0.7	700	70	70	–	170	50	2.7	0.660	12.600	0.585	10.230
Upper value	8.5	5.2	1.0	1.9	1700	350	210	–	420	140	9.5	8.700	32.950	9.370	30.730
Mean	–	–	–	–	–	–	–	–	–	–	–	2.856	18.660	2.830	19.368

*Data accidentally omitted from manuscript.
†CRP (titers) and CRP (mg-%) are negative at 1:1 dilution. Other ranges (g-%) are: 45. S-A-comp., 5.4-6.9; 7S-G-comp., 0.6-1.2; and 19S-M-comp., 0.12-0.44.

The following are the conclusions regarding the controls.

For Lawrence Phillips, predive values on three occasions showed elevations of total gamma globulin associated with concomitant increases in γG-globulin, γA-globulin, and γM-globulin. On one occasion (D - 30) a reduced transferrin value was obtained. These findings are compatible with an acute infectious disease on or about the D - 30 examination. No significant postdive abnormalities were observed.

For Ian Koblick and Gary Davis, no abnormalities were observed in any parameters throughout the observed periods.

No significant changes in any humoral or cellular immunologic parameters were identified relative to the dive interval.

A3.3.4 Blood Chemistries

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A3.3.4.1 Objectives, Measurements, and Methods

Specific objectives of the blood chemistry studies were (a) to determine the extent and time course of alterations in fluid and electrolyte balance and metabolism as reflected in selected biochemical constituents of blood and (b) to document the physical qualifications of the crew members for the mission and to detect problems which could require remedial or preventive action, thereby insuring optimum performance and comfort.

The following measurements were made (with the methods in parentheses): true serum glucose (autoanalyzer); blood urea nitrogen (autoanalyzer); creatine (autoanalyzer); Na, K, Mg, Ca, and Cl (flame photometry and atomic absorption spectrometer); phosphorus (autoanalyzer); SGOT (Babson method); alkaline phosphatase (Babson and Phillips method); creatine phosphatase (Nuttal and Wedin method); uric acid (autoanalyzer); bilirubin, total and direct (Diazzo method); serum and/or plasma osmolarity (freezing point osmoter); total red blood cell (RBC) and plasma lipid content* (gravimetric analysis); neutral lipid fractionation to include RBC and plasma: cholesterol, cholesterol ester,* free fatty acids,* monoglycerides,* diglycerides,* and triglycerides (thin-layer chromatography and gas-liquid chromatography); phospholipid fractionation to include RBC and plasma: phosphatidic acid, phosphatidyl serine, phosphatidyl ethanolamine, phosphatidyl choline, phosphatidyl inositide, spingomyelin, and lysolecithin (column chromatography, thin-layer chromatography, and gas-liquid chromatography); RBC and plasma steroids:* tocopherol, i.e., vitamin E, and vitamin A (fatty acid pattern on all neutral and gas-liquid chromatography phospholipids, RBC, and plasma listed above); and hydrocortisone (cortisol-binding globulin).

A3.3.4.2 Results

The results are given in Tables A15, A16, and A17.

A3.3.4.3 Discussion of Results

The clinical biochemical parameters sampled showed no diagnostic abnormalities; however, several significant trends are evident.

*The test was still in progress when this was written, and the results will be reported in other documents.

Table A15
Serum/Chemistry Results

Date Relative to D (Dive)	Glucose	Blood Urea Nitrogen	Bilirubin	Creatinine	Uric Acid	Alkaline Phosphatase	Creatine Phosphatase	LDH	SGOT	Na	K	Mg	Ca	Cl	PO_4	Cholesterol	Triglycerides	Osmolarity
Clifton (Diver)																		
D − 30	98	17	0.5	1.0	5.7	24	7	41	17	141.0	4.9	2.4	9.6	110	4.5	223	159	289
D − 11	78	15	0.2	1.0	5.6	28	28	52	24	142.5	4.4	2.6	9.6	109	3.8	223	82	288
D − 5	78	15	0.6	0.9	5.2	26	28	54	29	140.5	3.9	2.6	9.2	104	3.0	206	22	309
D + 14	78	19	0.4	1.1	5.1	32	34	54	28	141.0	4.0	2.5	9.1	102	3.9	187	65	286
D ± 21	80	15	0.9	1.0	5.5	32	30	61	30	141.5	4.2	2.5	9.8	104	3.2	235	103	286
D + 28	85	18	0.6	1.0	5.5	31	26	55	31	143.0	3.8	2.4	9.5	104	3.4	210	88	290
D + 35	82	18	0.9	1.1	5.9	29	26	25	32	143.5	4.1	2.5	10.2	104	3.8	247	126	288
D + 42	84	16	0.3	1.1	5.0	19	28	50	29	139.5	4.3	2.4	9.7	104	3.9	205	138	282
D + 49	86	22	0.6	1.1	6.1	30	20	55	31	143.0	4.5	2.5	10.0	105	3.2	235	115	295
D + 56	93	21	0.7	1.0	5.3	29	22	53	27	144.0	4.4	2.5	9.7	109	3.6	222	98	308
D + 60 (Bottom)	76	19	0.6	1.1	5.3	27	32	45	28	140.5	3.8	2.5	9.2	104	3.4	205	43	287
D + 60 (Surface)	82	19	0.7	1.0	5.1	25	18	51	38	140.0	4.4	2.5	9.0	102	3.4	205	85	284
Mahnken (Diver)																		
D − 30	81	17	0.9	1.0	4.9	29	11.9	51	25	140.0	4.4	2.4	9.7	108	3.4	155	125	292
D − 11	71	17	0.9	1.0	6.6	33	ND	61	32	143.0	3.9	2.3	10.5	110	3.2	168	50	290
D − 5	131	16	0.5	1.0	7.2	31	21	65	37	143.5	3.8	2.6	9.9	105	3.1	145	<10	302
D + 1	104	19	0.8	1.0	5.4	32	53	53	39	138.0	4.4	2.3	9.4	105	2.9	200	77	280
D + 14	74	17	0.5	1.0	5.3	40	15	57	31	143.0	4.2	2.5	10.0	103	3.6	148	39	293
D + 21	79	17	0.8	1.0	5.5	36	15	12	30	143.0	4.2	2.3	9.8	104	3.0	197	94	282
D + 28	87	18	0.7	1.0	5.5	39	17	49	33	144.0	4.4	2.4	10.1	106	3.6	175	75	296
D + 35	75	19	0.8	1.1	5.7	35	12	56	33	144.0	4.4	2.4	10.1	105	3.6	175	95	289
D + 42	82	16	0.6	1.1	5.4	20	17	53	33	143.5	4.5	2.5	10.1	105	3.2	153	90	291
D + 49	83	21	0.8	1.1	5.6	32	15	54	35	144.0	4.6	2.6	10.3	103	3.2	162	124	299
D + 56	93	20	0.6	1.0	5.1	33	ND	50	31	145.0	4.5	2.6	10.1	112	3.7	153	113	248
D + 60 (Bottom)	69	17	0.5	1.0	4.6	31	20	60	25	139.5	4.0	2.3	9.2	101	3.2	151	59	234
D + 60 (Surface)	85	20	0.9	1.0	5.4	29	19	55	41	140.0	4.0	2.5	9.5	104	3.2	153	50	234
Van Derwalker (Diver)																		
D − 30	86	15	1.2	1.1	5.6	18	3	43	16	141.0	4.0	2.4	10.0	104	4.7	144	128	288
D − 11	101	16	1.5	1.1	5.7	14	ND	46	29	138.0	3.7	2.2	9.8	105	2.8	145	34	283
D − 5	82	10	0.8	1.0	5.4	15	19	52	23	141.5	3.6	2.2	9.8	103	2.8	135	37	280
D + 1	97	21	1.1	1.1	5.4	13	5	46	31	137.5	3.8	2.3	9.5	99	3.3	171	72	280
D + 14	94	18	1.4	1.1	5.3	18	43	65	35	141.5	3.8	2.4	9.4	102	2.8	138	38	283
D + 21	81	17	1.5	1.0	5.7	16	18	48	24	142.0	4.1	2.1	9.2	101	2.9	164	101	287
D + 28	82	19	1.2	1.0	6.0	17	21	53	30	142.5	4.0	2.3	10.0	103	3.2	204	730	294
D + 35	74	18	1.9	1.2	6.4	16	14	49	26	142.5	4.0	2.2	9.8	102	3.6	175	72	286
D + 42	74	17	1.0	1.2	5.9	27	14	46	30	139.0	4.1	2.2	9.8	100	3.7	153	147	282
D + 49	81	20	0.9	1.1	6.1	16	11	51	35	141.0	4.2	2.3	9.6	103	3.1	175	139	293
D + 56	94	22	1.0	1.1	6.0	16	12	49	32	144.5	4.2	2.4	9.8	108	3.2	175	70	310
D + 60 (Bottom)	76	19	0.8	1.1	5.7	13	6	48	31	138.5	3.6	2.3	9.3	102	2.9	163	61	284
D + 60 (Surface)	90	18	1.7	1.1	5.7	13	5	47	35	139.0	4.0	2.4	9.6	103	3.0	146	32	282
Waller (Diver)																		
D − 30	89	14	0.5	1.1	5.2	15	5	40	22	142.0	5.0	2.5	9.9	110	4.2	195	220	286
D − 11	84	13	0.8	1.1	5.8	17	16	45	20	142.5	3.9	2.5	9.8	106	2.9	194	40	280
D − 5	67	14	0.3	1.0	5.5	17	16	37	23	140.0	4.0	2.3	9.5	108	2.8	205	ND	276
D + 1	97	17	1.0	1.1	6.0	15	8	44	28	136.5	3.8	2.6	9.6	100	3.1	142	80	274
D + 14	76	21	ND	1.1	4.9	8	12	45	29	143.0	3.8	ND	9.4	105	ND	200	73	284
D + 21	80	14	0.7	1.0	5.9	17	10	75	55	143.0	4.4	2.4	9.7	106	3.3	231	101	284
D + 28	84	18	0.6	1.0	5.8	16	7	52	35	143.0	4.0	2.5	9.7	104	3.2	240	ND	297
D + 35	75	19	0.7	1.1	5.8	16	16	58	35	142.5	4.0	2.4	10.2	103	4.1	236	126	285
D + 42	80	15	0.3	1.1	5.3	20	14	43	26	142.5	4.0	2.4	9.6	105	4.4	215	117	287
D + 49	88	18	0.9	1.0	6.1	18	12	66	36	142.5	4.2	2.4	10.0	105	3.4	225	139	295
D + 56	84	22	0.7	1.0	5.7	18	20	48	39	144.5	4.0	2.6	9.9	108	3.1	195	85	297
D + 60 (Bottom)	78	18	0.5	1.0	5.5	16	18	50	26	138.0	3.3	2.5	9.4	100	2.9	203	48	282
D + 60 (Surface)	88	16	0.3	1.0	5.7	17	8	47	22	140.5	3.8	2.6	9.4	106	3.4	203	ND	280

(Table continues)

Table A15 (Continued)

Date Relative to D (Dive)	Glucose	Blood Urea Nitrogen	Bilirubin	Creatinine	Uric Acid	Alkaline Phosphatase	Creatine Phosphatase	LDH	SGOT	Na	K	Mg	Ca	Cl	PO_4	Cholesterol	Triglycerides	Osmolarity
Davis (Control)																		
D−30	105	14	0.4	1.2	6.2	20	104	57	79	142.0	4.8	2.4	10.6	106	3.7	172	85	285
D−11	74	17	0.5	1.1	6.1	20	23	51	32	141.0	4.0	2.4	10.0	105	2.9	185	41	293
D−5	84	15	0.8	1.0	5.7	21	53	57	24	140.5	3.6	2.3	9.9	103	3.0	176	10	280
D+14	82	21	0.4	1.3	6.0	24	50	57	26	144.0	4.3	2.4	10.2	104	3.2	153	39	294
D+21	84	16	0.9	1.1	6.0	24	30	69	43	142.0	4.6	2.2	10.2	104	2.6	168	79	284
D+28	92	13	0.7	1.2	6.3	25	58	57	30	141.5	3.8	2.0	10.2	104	2.6	168	QNS	284
D+35	82	18	0.7	1.1	6.2	19	23	55	38	143.0	4.2	2.3	10.1	105	3.4	177	101	292
D+42	88	17	0.5	1.1	5.9	21	32	54	33	141.0	4.0	2.1	10.1	104	2.8	183	121	285
D+49	77	16	0.6	1.1	5.0	20	24	57	34	141.5	3.8	2.2	9.9	103	3.4	173	131	286
D+56	95	18	0.5	1.1	5.7	24	20	71	35	139.5	4.4	2.3	10.3	107	3.1	183	98	293
D+60 (Bottom)	85	16	0.4	1.2	6.1	25	19	44	28	143.5	3.6	2.2	9.6	107	2.1	153	105	288
D+60 (Surface)	96	14	0.7	1.1	5.6	22	16	48	34	140.5	4.0	2.2	9.6	106	2.6	153	68	283
Koblick (Control)																		
D−30	106	14	0.2	1.2	6.0	20	16	36	18	139.5	4.6	2.1	9.9	105	3.6	164	148	289
D−11	80	16	1.3	1.2	6.0	21	27	47	24	140.0	3.6	2.0	9.6	101	2.7	192	34	282
D−5	90	14	0.5	1.1	6.3	20	32	50	32	141.5	4.3	2.2	9.8	104	3.6	178	35	288
D+14	90	15	1.2	1.2	5.9	22	50	49	36	142.5	4.4	2.2	9.7	104	3.3	193	24	295
D+21	88	17	0.6	1.1	6.0	21	30	55	34	141.5	4.9	2.1	10.0	104	3.4	184	75	282
D+28	91	17	0.6	1.2	6.5	22	23	58	33	140.5	4.6	2.0	10.3	102	3.0	204	24	285
D+35	78	18	1.2	1.2	5.7	29	42	43	34	140.0	4.2	2.1	9.9	100	3.7	210	96	285
D+42	86	16	1.2	1.2	6.3	20	38	50	35	140.0	4.2	4.2	10.1	103	3.5	213	115	284
D+49	75	18	0.8	1.2	6.5	18	46	51	38	141.0	3.9	2.2	9.8	103	4.5	185	120	287
D+56	113	19	0.7	1.1	6.6	19	28	59	35	139.0	4.6	2.1	9.9	108	3.3	193	118	299
D+60 (Bottom)	91	18	0.8	1.2	6.0	16	32	41	26	141.5	4.1	2.3	9.5	106	3.4	182	65	284
D+60 (Surface)	82	13	0.6	1.1	6.8	18	32	49	32	139.0	4.5	2.1	9.5	104	2.5	185	131	279
Phillips (Control)																		
D−30	112	15	<0.8	0.8	4.1	20	7	43	20	140.0	5.0	2.4	9.9	109	3.4	109	280	QNS
D−11	88	14	0.6	0.9	5.5	22	21	50	11	141.5	4.3	2.7	9.8	108	3.8	176	218	289
D−5	84	14	ND	0.7	4.9	26	9	59	31	140.0	3.8	2.6	9.6	106	3.4	186	285	284
D+14	78	17	0.9	1.0	5.4	28	17	56	31	142.0	4.2	2.6	9.7	102	4.1	140	220	284
D+21	97	17	1.2	0.8	5.3	26	23	52	28	140.5	3.9	2.4	9.8	106	3.4	164	226	284
D+28	85	16	0.7	0.9	5.3	25	13	59	21	140.0	4.2	2.4	10.2	103	3.8	178	QNS	293
D+35	79	14	0.9	0.9	5.2	21	18	56	26	140.5	3.9	2.5	9.7	106	3.9	167	357	286
D+42	74	15	0.5	0.9	4.7	26	15	47	26	141.0	3.8	2.4	9.5	105	3.7	157	>250	288
D+49	73	15	0.8	1.0	5.1	22	6	54	33	141.0	3.9	2.4	9.8	104	4.3	158	162	287
D+56	99	20	0.8	0.9	5.3	23	13	69	33	138.5	4.4	2.5	10.1	108	3.9	163	125	298
D+60 (Bottom)	88	18	1.0	1.0	5.3	20	15	49	31	141.5	4.0	2.6	9.4	107	4.0	148	141	287
D+60 (Surface)	88	18	1.2	0.9	5.6	20	12	48	29	139.0	4.2	2.5	9.8	107	3.3	151	115	284

The blood urea nitrogen (BUN) appears generally to increase over the last weeks of the dive interval, with return toward predive control values after decompression (Table A15). Rising BUN values are often associated with prerenal diversion of water, increased protein catabolism, and impaired renal function. No evidence of renal impairment is found in the associated chemistry data, and dehydration is not indicated. Increased protein catabolism, or dietary factors are probably contributory.

Serum glutamic oxalacetic transaminase (SGOT) levels showed an unexplained increase during the dive interval (Table A15). Since these elevations were not associated with other enzyme shifts, this isolated finding is of no diagnostic importance.

Table A16
Electrophoresis Results

Date Relative to D (Dive)	Lipo α_1	Pre β	β	LDH					TP	Albumin	α_1	α_2	β	γ
				1	2	3	4	5						
Clifton (Diver)														
D - 30	27	22	46	23	29	22	3	1	6.8	4.1	0.2	0.6	0.8	1.1
D - 11	30	24	47	35	19	34	7	4	7.4	4.5	0.1	0.6	0.8	1.3
D - 5	26	17	59	25	26	29	14	4	6.9	4.2	0.1	0.7	0.6	1.2
D + 14	ND	ND	ND	28	37	26	6	4	7.3	4.4	0.1	0.8	0.8	1.2
D + 21	31	23	46	27	26	27	7	12	7.7	4.6	0.2	0.8	0.9	1.2
D + 28	22	23	55	36	32	20	4	7	7.3	4.3	0.1	0.9	0.8	1.2
D + 35	27	16	57	ND	ND	ND	ND	ND	7.7	4.4	0.1	0.7	0.7	1.7
D + 42	ND	ND	ND	ND	ND	ND	ND	ND	7.4	4.6	0.1	0.6	0.6	1.3
D + 49	28	13	60	25	35	28	8	5	7.7	4.6	0.4	0.7	0.8	1.3
D + 56	27	18	55	26	34	20	11	9	7.8	5.2	0.1	0.6	0.7	1.2
D + 60 (bottom)	ND	ND	ND	ND	ND	ND	ND	ND	7.2	4.5	0.2	0.6	0.7	1.2
D + 60 (surface)	ND	ND	ND	ND	ND	ND	ND	ND	7.0	4.5	0.2	0.6	0.6	1.1
Mahnken (Diver)														
D - 30	26	23	39	19	29	21	3	5	6.6	4.5	0.2	0.4	0.6	0.8
D - 11	40	21	37	28	39	28	6	2	7.2	4.7	0.2	0.5	0.8	1.0
D - 5	39	19	44	22	30	38	5	4	7.0	4.3	0.2	0.7	0.7	1.1
D + 1	ND	ND	ND	ND	ND	ND	ND	ND	6.6	4.3	0.2	0.5	0.6	1.0
D + 14	ND	ND	ND	27	39	26	4	3	7.3	5.0	0.1	0.6	0.7	0.9
D + 21	26	23	55	25	36	19	10	10	6.9	4.3	0.2	0.6	0.8	1.0
D + 28	30	23	45	34	38	21	3	4	7.0	4.3	0.1	0.7	0.8	1.0
D + 35	27	22	51	ND	ND	ND	ND	ND	7.1	4.5	0.1	0.9	0.6	1.0
D + 42	ND	ND	ND	ND	ND	ND	ND	ND	7.0	4.5	0.2	0.7	0.6	1.0
D + 49	34	21	44	23	37	22	11	8	7.3	4.7	0.4	0.6	0.6	1.0
D + 56	28	16	56	24	39	20	9	8	7.1	4.8	0.1	0.7	0.5	1.0
D + 60 (bottom)	ND	ND	ND	26	42	17	9	6	6.8	4.4	0.2	0.4	0.6	1.1
D + 60 (surface)	ND	ND	ND	ND	ND	ND	ND	ND	6.5	4.2	0.2	0.5	0.6	1.0
Van Derwalker (Diver)														
D - 30	33	17	45	22	35	29	8	2	7.9	4.8	0.2	0.5	0.8	1.5
D - 11	31	23	50	30	28	38	5	3	7.5	4.5	0.1	0.5	0.8	1.5
D - 5	32	19	49	27	28	32	4	9	7.0	4.1	0.1	0.4	0.7	1.6
D + 1	ND	ND	ND	ND	ND	ND	ND	ND	7.8	4.6	0.3	0.5	0.6	1.8
D + 14	ND	ND	ND	25	37	29	5	4	7.5	4.5	0.2	0.6	0.8	1.5
D + 21	36	30	38	23	42	16	10	9	7.2	4.2	0.2	0.6	0.8	1.4
D + 28	27	25	49	37	31	20	4	8	7.9	4.4	0.2	0.7	0.9	1.7
D + 35	32	18	50	ND	ND	ND	ND	ND	7.8	4.5	0.1	0.7	0.8	1.7
D + 42	ND	ND	ND	ND	ND	ND	ND	ND	7.9	4.8	0.2	0.5	0.6	1.7
D + 49	34	19	46	24	35	22	9	10	7.8	4.5	0.4	0.6	0.8	1.6
D + 56	33	16	50	23	35	25	9	9	7.7	4.9	0.2	0.4	0.5	1.5
D + 60 (bottom)	ND	ND	ND	17	36	27	14	6	7.4	4.6	0.2	0.4	0.6	1.6
D + 60 (surface)	ND	ND	ND	ND	ND	ND	ND	ND	7.3	4.4	0.2	0.5	0.6	1.6

(Table continues)

Table A16 (Continued)

Date Relative to D (Dive)	Lipo α_1	Pre β	β	LDH 1	LDH 2	LDH 3	LDH 4	LDH 5	TP	Albumin	α_1	α_2	β	γ
Waller (Diver)														
D - 30	23	17	56	21	33	22	4	1	6.9	4.0	0.3	0.6	1.0	1.0
D - 11	26	21	53	31	28	34	5	3	7.5	4.5	0.2	0.7	0.8	1.2
D - 5	34	14	53	32	23	30	10	0	7.1	4.5	0.2	0.6	0.8	1.1
D + 1	ND	ND	ND	ND	ND	ND	ND	ND	6.8	4.3	0.2	0.6	0.7	1.0
D + 14	ND	ND	ND	25	37	26	7	5	7.2	4.7	0.2	0.6	0.8	0.9
D + 21	22	24	55	27	33	24	6	9	7.1	4.3	0.2	0.7	0.8	1.0
D + 28	20	21	58	28	33	23	6	10	7.1	4.3	0.2	0.7	0.9	1.0
D + 35	23	21	56	ND	ND	ND	ND	ND	7.8	4.7	0.2	0.8	0.9	1.2
D + 42	ND	ND	ND	ND	ND	ND	ND	ND	7.1	4.7	0.1	0.6	0.7	1.0
D + 49	25	19	55	24	36	23	10	8	7.7	4.8	0.4	0.8	0.8	1.0
D + 56	29	15	56	22	36	18	13	10	7.6	4.8	0.1	0.7	0.8	1.2
D + 60 (bottom)	ND	ND	ND	18	36	27	14	5	7.2	4.7	0.2	0.6	0.7	1.1
D + 60 (surface)														
Davis (Control)														
D - 30	25	20	55	17	27	30	4	1	6.8	4.1	0.3	0.7	0.7	1.0
D - 11	25	22	53	34	29	29	5	4	7.0	4.5	0.2	0.5	0.7	1.0
D - 5	22	27	49	24	34	23	11	6	6.7	4.4	0.1	0.6	0.6	0.9
D + 14	ND	ND	ND	26	35	21	9	9	7.1	4.5	0.2	0.6	0.8	1.0
D + 21	28	30	44	27	37	27	5	4	7.2	4.6	0.3	0.6	0.8	0.9
D + 28	26	18	55	28	33	22	7	11	6.7	4.3	0.2	0.6	0.7	0.9
D + 35	19	18	63	ND	ND	ND	ND	ND	6.8	4.4	0.2	0.6	0.7	0.9
D + 42	ND	ND	ND	ND	ND	ND	ND	ND	7.2	4.7	0.2	0.6	0.6	1.1
D + 49	20	19	61	24	35	23	9	9	6.8	4.0	0.4	0.6	0.8	1.0
D + 56	29	22	49	23	33	28	9	8	7.4	5.0	0.2	0.6	0.8	0.9
D + 60 (bottom)	ND	ND	ND	22	42	24	11	9	6.6	4.2	0.2	0.5	0.7	1.0
D + 60 (surface)	ND	ND	ND	ND	ND	ND	ND	ND	6.2	3.8	0.2	0.5	0.8	0.9
Koblick (Control)														
D - 30	31	23	45	27	24	23	5	2	6.8	3.9	0.4	0.6	0.6	1.2
D - 11	45	14	41	29	30	33	7	4	7.2	4.7	0.2	0.5	0.8	1.1
D - 5	29	22	50	35	31	24	5	5	7.1	4.6	0.1	0.5	0.7	1.2
D + 14	ND	ND	ND	27	33	26	9	5	7.3	4.7	0.1	0.5	0.7	1.2
D + 21	37	27	35	22	33	28	9	8	7.1	4.2	0.2	0.6	0.9	1.1
D + 28	39	21	40	31	32	21	7	9	7.2	4.2	0.2	0.8	1.0	1.1
D + 35	33	15	52	ND	ND	ND	ND	ND	6.9	4.4	0.1	0.5	0.7	1.2
D + 42	ND	ND	ND	ND	ND	ND	ND	ND	7.7	5.0	0.1	0.4	0.8	1.4
D + 49	41	20	39	22	33	25	9	11	6.7	4.1	0.4	0.5	0.6	1.1
D + 56	37	17	46	28	36	20	7	8	7.6	5.0	0.2	0.5	0.8	1.2
D + 60 (bottom)	ND	ND	ND	19	39	22	14	6	7.3	4.6	0.2	0.4	0.6	1.4
D + 60 (surface)	ND	ND	ND	ND	ND	ND	ND	ND	6.9	4.2	0.2	0.5	0.8	1.2
Phillips (Control)														
D - 30	20	25	52	14	32	22	6	2	7.2	3.7	0.3	0.8	0.7	1.6
D - 11	24	30	46	30	35	24	7	5	7.9	4.9	0.1	0.4	0.9	1.5
D - 5	15	24	62	17	36	25	15	5	7.6	4.6	0.1	0.6	0.8	1.5
D + 14	ND	ND	ND	27	37	21	8	6	7.8	4.9	0.2	0.6	0.8	1.4
D + 21	25	61	20	22	38	18	12	11	7.5	4.7	0.1	0.6	0.8	1.3
D + 28	25	44	30	26	31	32	6	5	7.5	4.6	0.2	0.6	0.8	1.4
D + 35	18	13	69	ND	ND	ND	ND	ND	7.5	4.6	0.2	0.7	0.7	1.3
D + 42	ND	ND	ND	ND	ND	ND	ND	ND	6.8	4.2	0.2	0.6	0.5	1.3
D + 49	27	13	60	21	35	25	10	9	7.1	4.3	0.4	0.5	0.7	1.2
D + 56	29	39	32	22	35	22	12	9	7.9	5.3	0.1	0.4	0.7	1.3
D + 60 (bottom)	ND	ND	ND	25	39	23	11	3	6.8	4.5	0.1	0.5	0.6	1.2
D + 60 (surface)	ND	ND	ND	ND	ND	ND	ND	ND	6.9	4.2	0.2	0.5	0.6	1.4

Table A17
Hydrocortisone Results

Date Relative to D (Dive)	Plasma Hydrocortisone* (μg/100 ml)						
	Clifton	Mahnken	Van Der-walker	Waller	Davis	Koblick	Phillips
D - 30	9.8	11.5	11.2	9.5	10.8	8.2	15.3
D - 11	21.0	4.8	24.6	8.5	14.6	11.9	20.0
D - 5	10.4	10.5	13.0	15.9	10.2	12.0	7.0
D + 28	19.0	19.4	19.8	19.0	9.4	10.0	6.2
D + 56	12.2	9.0	15.5	14.5	10.1	9.2	9.5
D + 60 (bottom)	18.5	16.8	12.8	16.0	10.8	11.3	11.0
D + 60 (surface)	10.0	9.8	11.0	6.2	11.8	23.2	20.8
D + 61	24.8	17.0	23.5	30.8	6.8	6.0	15.0

*Normal values = 10 to 20 μg/100 ml; 1 standard deviation = 0.5.

The plasma hydrocortisone values (Table A17) demonstrate considerable interindividual variability. Although there are no abnormal results, certain trends are of interest and merit further discussion and investigation. There is a suggestion of a decrease in values during the in-dive phase (between the fourth and eighth week) on all four divers which does not appear in the control values. The significance of this is not apparent, although one explanation could be the operation of an adaptative process. The apparent increase subsequent to the eighth-week sample could be due to the "expectation" excitement and anticipation of leaving the habitat.

Another interesting trend is the slight decrease in values seen from the sample drawn on the bottom and that drawn immediately after decompression. This same sort of trend has been noted, but unexplained, in the Apollo flights subsequent to splashdown. Further work is being planned to consider the control mechanisms involved in this neuroendocrine process.

A3.3.5 Instructions to Aquanauts on Blood Drawing and Processing

The following were the instructions given to the aquanauts concerning blood drawing and processing:

A. Basic principles of blood drawing

1. Set the crewman's arm in a comfortable position, fully extended.

2. Place a tourniquet on the upper arm — not too tight.

3. Have the crewman pump his hand for several seconds or until a prominent vein appears.

4. Cleanse the area around the vein selected.

5. Maintaining the sterility of the needle, enter the vein through the area cleansed and aspirate the syringe until blood freely flows. (If on two successive occasions, the vein is not entered, have another crewman perform the venapuncture.)

6. Then RELEASE TOURNIQUET and draw an appropriate sample into the syringe (see the sample chart).

Sample Chart

Indive Week	Date	Volume of Draw (ml)	Indive Week	Date	Volume of Draw (ml)
2	2/26/69	24	6	3/26/69	31
3	3/05/69	31	7	4/02/69	24
4	3/12/69	34	8	4/09/69	34
5	3/19/69	24			

7. When a sample has been acquired, place a clean dry sponge over the needle puncture site and withdraw the needle quickly.

8. Distribute the blood into the tubes provided. Invert the tubes gently ten times for adequate anticoagulation.

B. Blood Processings

All samples tubes are provided and prelabeled for each weekly sampling period. Different amounts are required on various weeks, with different anticoagulants. For sample tube selection, each tube is color-coded. Select a set of color-coded tubes according to the sample chart. Fill each tube to the red mark, and gently oscillate it ten times. *Push a needle through each tube top, thereby providing a vent for decompression!*

A3.4 Microbiology of the Aquanauts and Their Environment

A3.4.1 Introduction

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

The relationship between man and his environment is important in maintaining a proper balance among those microorganisms which comprise his indigenous microflora and hence his health and well being. Slight changes in the environment may reflect themselves as an alteration in this balance. The conditions necessary to sustain the Tektite I habitat in the submerged state from an engineering standpoint, the confinement of the aquanauts to the habitat and marine environment, and the interactions of the aquanauts, both with each other and the environment are major factors, each with its myriad of minor interacting elements, that can affect man's indigenous microflora. A study of the effect of these conditions in prolonged submergence is necessary to define the effects of such an environment on the microorganisms associated with the aquanauts.

An extensive study was carried out to determine the types, the numbers, and the frequency of occurrence of microorganisms in five body sites, two areas on the interior surface of the habitat, and the air within the habitat during the 59-day period of the Tektite I program. Samples of microbiological analysis were taken before and during the period of submergence. The continued sampling through the entire program allows for evaluation of the various experimental conditions in terms of their influence on the microflora.

A3.4.2 General Sampling Procedures

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

A3.4.2.1 Aquanauts

To determine changes in the bacterial and fungal flora associated with the aquanauts samples were collected at various body sites with the aid of saline-wetted, cotton-tipped sterile swabs. The sites sampled were the forearm (approximately 9 sq in.), behind the knee (approximately 5 sq in.), the throat, the ear, and the rectum (immediately after defecation). The swab tips were broken off in 1-dram vials containing 1.2 ml of media composed of brain-heart infusion broth (Difco) containing 10% horse serum and 5% glycerol. The sampling of each aquanaut was made twice weekly, on Wednesday and Saturday, in the morning before entry into the water and not before 4 hours had elapsed since showering.

Samples were also obtained from the throat and rectum of each aquanaut for virological analysis. The swabs were collected as above, and for each sample one swab was rinsed in a 1-dram vial with 1.2 ml of veal infusion broth (Difco) containing 0.5% bovine albumin and a second swab was placed in a test tube containing the charcoal viral transport medium (CVTR) of Leibovitz.* Sampling was performed on a 10-day schedule to coincide with the bacterial and fungal sampling.

Serum samples were obtained from each aquanaut before the start of the program and on a weekly basis during the dive. The serum was collected as part of the hematology program. The sera were maintained at -60°C and sent to the Naval Biological Laboratory (NBL) with the virus samples.

A3.4.2.2 Habitat

To determine if changes in population or an accumulation of bacteria and fungi occurred during the 59 days of the program the habitat walls were sampled at intervals in the crew quarters and in the wet lab. These sites were sampled on the same schedule and handled in a similar manner as the samples obtained from the aquanauts. The site sampled had not previously been swabbed in order that an accumulation of bacteria could be detected. The sites are depicted in Figs. A28 and A29 and represent an area of 8 sq in.

At the completion of the program, small patches of rug were removed from the various spaces in the habitat and sent to the surface for mycological examination.

The swabs and vials for aquanaut and habitat sampling were transferred to the aquanauts on the afternoon of the day prior to sampling. In the event that the rectal sample was collected during the day or night before the sample day, the vial was stored in the refrigerator until returned to the surface with those samples obtained at the scheduled time.

A3.4.2.3 Aerobiology

Habitat air was sampled four times weekly in the wet lab using two six-stage Andersen samplers.† Half-strength tripticase soy agar (TSA), a general-purpose medium, was

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*A. Leibovitz, "A Transport Medium for Diagnostic Virology," Proc. Soc. Exptl. Biol. Med. 131, 127-130 (1969).

†A. Andersen, "A New Sampler for the Collection, Sizing and Enumeration of Viable Airborne Particles," J. Bacteriol. 76, 471-484 (1958).

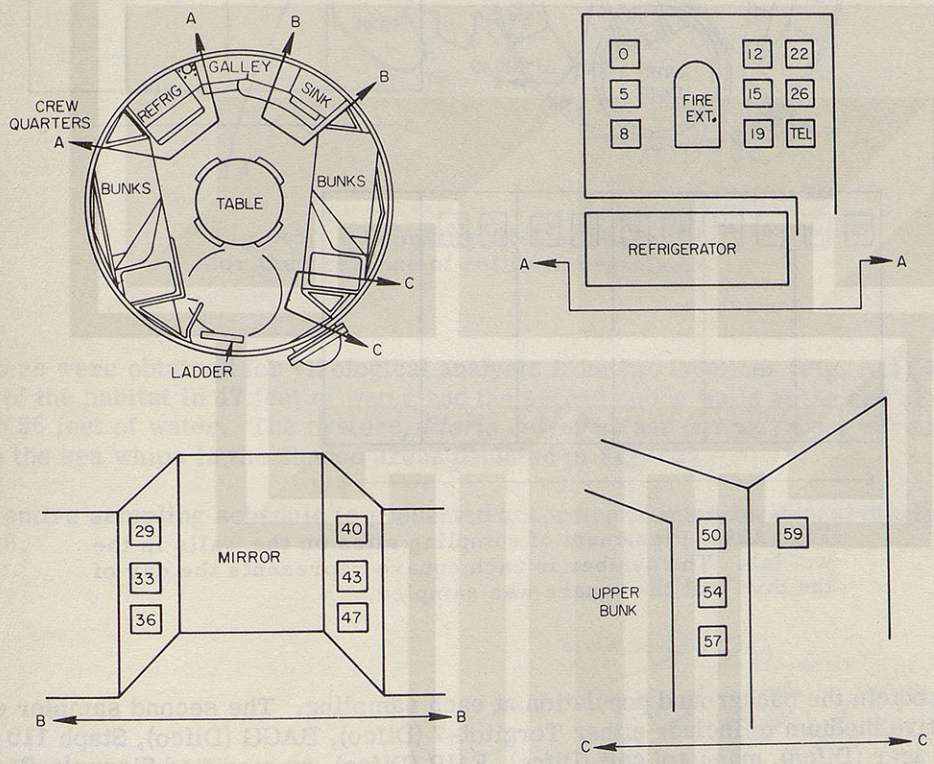

Fig. A28 - Placement of sampling sites on the walls of the crew quarters. The number in each square represents the day of the dive that the square was sampled.

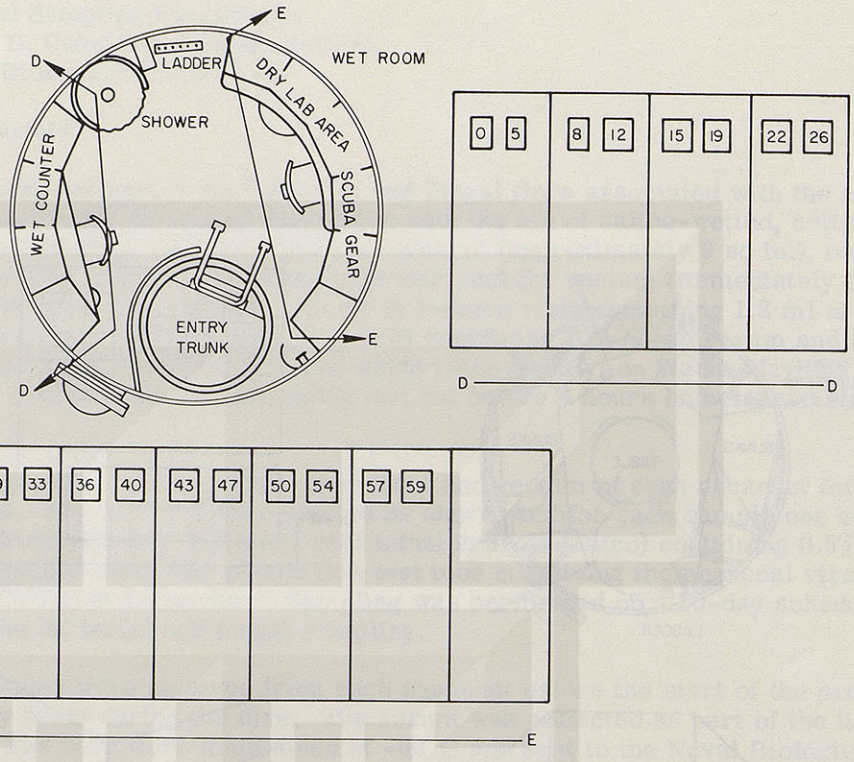

Fig. A29 - Placement of sampling sites on the walls in the wet lab. The number in each square represents the day of the dive that the square was sampled.

used to obtain the background population at each sampling. The second sampler employed a selective medium to include either Tergitol-7 (Difco), BAGG (Difco), Staph 110 (Difco), marine agar (Difco), mannotol salt (Difco), EMB (Difco), or modified Sierra's *Pseudomonas* medium* on a rotational basis. The Andersen samplers were loaded with the appropriate media on the surface and lowered to the aquanauts in sealed containers just prior to the taking of the air sample. They were taken by the aquanauts for a predetermined period of time, returned to the surface, and subsequently taken to the base camp for incubation. The schedule for air sampling was on Tuesday and Friday evenings and Wednesday and Saturday mornings.

A3.4.2.4 Marine Microbiology

Sea-water samples were collected from depths greater than 30 feet at four sites in the area of the habitat using the Cobet water sampler (Hydro Products) (Fig. A30). The sea water was analyzed by the membrane filter technique as outlined in "Standard Methods for the Examination of Water and Waste-Water"† using m-Endo Medium (Difco) for coliform enumeration.

*A. H. Wahba and J. H. Darrell, "The Identification of Atypical Strains of Pseudomonas aeruginosa," J. Gen. Microbiol. 38, 329-342 (1965).

†"Standard Methods for the Examination of Water and Waste-Water," 12th edition, Amer. Pub. Health Assoc. Inc., New York, 1965.

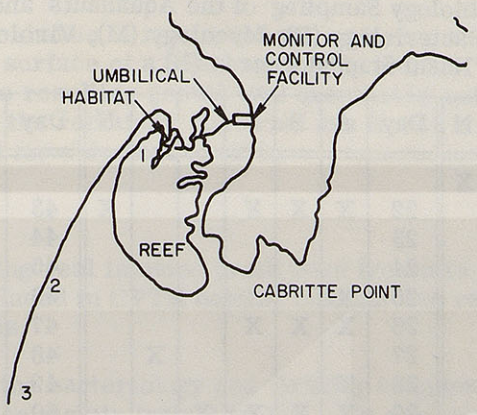

Fig. A30 - Sampling sites of sea water for the presence of coliform organisms

Oysters were obtained for virological analysis from two locations (Fig. A31), one in the area of the habitat in 47 feet of water and the second on the walls at the end of canyon 1 in 40 to 55 feet of water. The oysters, *Pteria colymbus* and *Ostrea frons*, were associated with the sea whips in the shaded areas depicted in Fig. A31.

The entire sampling schedule is presented according to microbiological discipline in Table A18.

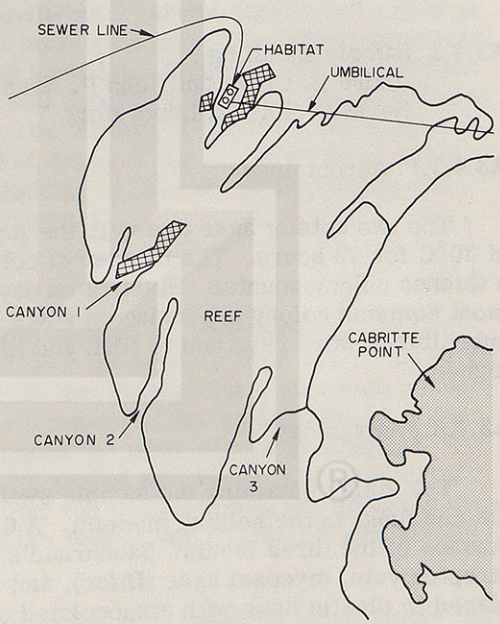

Fig. A31 - Collection sites of oysters for virological analysis. A single sample was collected from one of the two shaded areas near the habitat, and a second sample was collected from canyon 1.

Table A18
Schedule of Microbiology Sampling of the Aquanauts and Environment: Aerobiology (N), Bacteriology (B), Mycology (M), Virology (V), Marine Microbiology (P), Nasal Staphylococcus (N)

Day	A	B	M	V	P	N	Day	A	B	M	V	P	N	Day	A	B	M	V	P	N
0	X	X	X	X	X	X														
1							22	X	X	X			X	43						
2							23							44					X	
3					X		24							45						
4	X						25	X						46	X					
5	X	X	X				26	X	X	X				47	X	X	X	X		
6							27					X		48					X	
7	X				X		28	X						49	X					
8	X	X	X	X			29	X	X	X	X			50	X	X	X			
9					X		30					X		51					X	
10							31							52						
11	X						32	X						53	X					
12	X	X	X				33	X	X	X				54	X	X	X	X		
13					X		34							55					X	
14	X						35	X				X		56	X					
15	X	X	X				36	X	X	X	X			57	X	X	X			
16					X		37					X		58					X	
17							38							59	X	X	X	X		X
18	X						39	X						60						
19	X	X	X	X			40	X	X	X			X	61	X					
20					X		41					X								
21	X						42													

A3.4.3 Sample Handling
Andre B. Cobet and John P. Hresko,
Naval Biological Laboratory

A3.4.3.1 Aerobiology

The two sets of agar plates in the Andersen samplers were removed and incubated at 30°C for 72 hours. The number of colonies of bacteria and fungi were recorded using a Quebec colony counter. Starting on day 35 and on each subsequent sampling day the most common colony appearing on stage 4 of the TSA sample-set was subcultured. The subcultures were returned to NBL and identified using standard bacteriological techniques.

A3.4.3.2 Mycology

The vials containing the sample swabs were shaken vigorously to suspend the bacteria and fungi in the holding medium. A 0.1-ml aliquot of the sample was plated to the surface of the three media: Sabouraud's glucose agar (Difco) containing penicillin and streptomycin, mycosel agar (Difco), and malt agar (Difco). The inoculated plates were placed in plastic bags with uninoculated control plates, packaged, and sent via air mail to NBL.

On selected occasions, sea water collected in the area of the habitat at site 1 (Fig. A30) was membrane-filtered in 100-ml aliquots, and the filter was placed on the surface of the three media above and sent to NBL.

A3.4.3.3 Bacteriology

The above vials were shaken to resuspend the material, and with a calibrated loop 0.01 ml was plated to the surface of a blood agar plate (BAP). The BAP was incubated at 37°C for 24 hours, and the resulting growth was quantitated and recorded for on-site evaluation. The vial with the remaining material was frozen at -60°C in an NBL miniature deepfreezer and held for shipment to NBL.

A3.4.3.4 Virology

Sample vials containing veal infusion broth were frozen to -60°C in an NBL miniature deepfreezer. The swab placed in CVTR holding medium was returned to NBL by air mail with the mycology samples.

The vials containing the bacteriology and virology samples were sealed in No. 10 tin cans and shipped on dry ice via air freight to NBL. On arrival the tins were distributed to the respective investigators, who maintained the samples at -70°C until analysis was started.

A3.4.4 Aerobiology

R. L. Dimmick and Andre B. Cobet, Naval Biological Laboratory

A3.4.4.1 Introduction

The microbial flora of an individual is composed of a variety of bacteria, viruses, and fungi. This flora can be shed into the environment in quantity enough to be hazardous. Fortunately the predominant types of microorganisms comprising the individual's flora are harmless, enjoying a commensal existence with the host. A few microbial species are potential pathogens, eliciting infection when the hosts' defense mechanisms decline.

One important method of transfer of microorganisms from one person to another is aerosolization. This may result from a variety of conditions including motion of objects, both inanimate and human, or through coughing and sneezing. In a confined environment such as that of Tektite I, the airborne microflora will depend on an interrelation between the activity of the divers, input from external sources, effects of humidity and temperature, and removal by air filtration and settling. The assay of the air microflora in the Tektite I habitat was an attempt to gain an understanding of the rate of dispersion and equilibrium of the microbial population in the environment.

A3.4.4.2 Results

The number of airborne bacteria growing on the general purpose medium (TSA) increased from 3.5/ft^3 prior to the entry of the aquanauts on day 1 to over 100/ft^3 on three occasions, days 19, 22, and 42 (Fig. A32). A best-fit line through the data points reveals a continued increase in the number of organisms recovered to day 42 followed by a decrease. The values range from a low of 7.7 organisms/ft^3 after day 18 to a high of 189/ft^3 on day 42. The average count for the 59-day period was 44/ft^3 (standard error of mean = 10.1). The air in the habitat 48 hours after the completion of the program contained 0.3 organism/ft^3. There was no activity in the habitat during this period, although the engineering systems remained in operation.

The number of organisms which grew on marine agar was steady at about 10/ft^3 through day 33. An increase is noted on day 40, with a peak on day 46 at 483/ft^3 and decline at day 57. These organisms are capable of growing in a low-nutrient medium with sea-water salts incorporated in the formulation. This does not mean these organisms are necessarily marine organisms, but they represent a different population than that found on TSA.

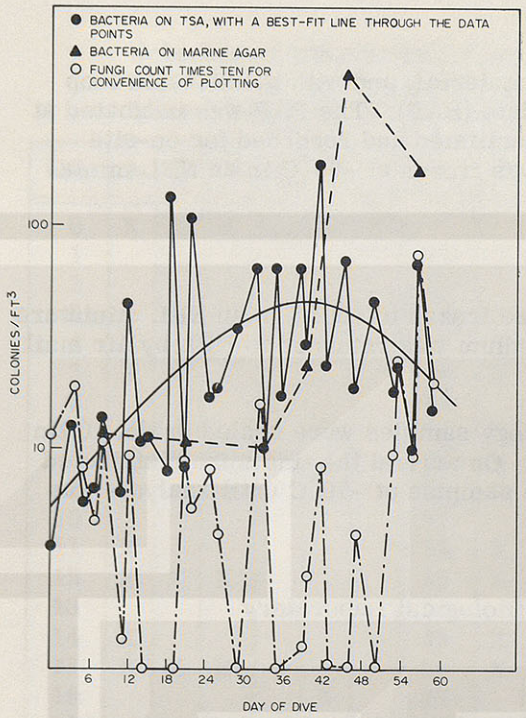

Fig. A32 - Concentration of airborne microbes in the Tektite I habitat

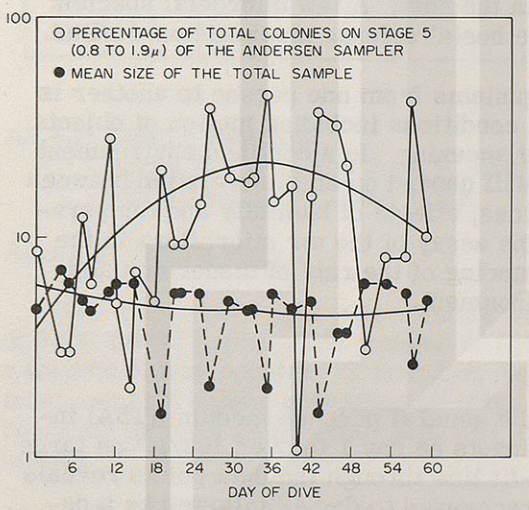

Fig. A33 - Particle size of airborne microbes in the Tektite habitat. Best-fit lines are shown.

The number of airborne fungi were increased during six peak periods. The data points in Fig. A32 are presented at 10 times the value found. The habitat had a moderate level of fungi during the first 12 days followed by moderate peaks from day 21 through day 25, at day 32, and at day 42, a low peak at day 47, and a high level from day 53 through day 59. The fungi count was always less than 10/ft^3 on the TSA medium.

The average particle size was 4.6 μm with a range from 7.0 μm on day 4 down to 1.5 μm on two occasions (Fig. A33). On 11 occasions the particle size was less than 5.0 μm. This is important, since it has been shown that particles less than 5.0 μm are capable of penetrating the alveolar spaces of the lung.*

The average percentage of bacteria found on stage 5, compared with the total number of bacteria on all six stages of the samples was 13%. These values ranged from a low of 1% on day 40 to a high of 43% on day 35. The fifth stage of the Andersen sampler retains particles in the size range 1.9 to 0.8 μm.† Here again the size is important as related to the capability of lung penetration of the particle. The trend showed an increase in percentage to day 35 followed by a decrease.

The first few days of sampling revealed a wide variety of colony types but low numbers of organisms on the agar plates. As the program progressed, the number of colonies increased and the variety of colonies decreased. No attempt was made to identify the organisms appearing on the various stages. It was reasoned that with the reduction in variety there may be an emergence of a single group or species of bacteria. On day 35, and from day 39 through day 57, the most common colony type was subcultured from stage 4 of the sampler and later identified. The 12 samples on further analysis produced 15

*H. A. Druett, "The Inhalation and Retention of Particles in the Human Respiratory System," in P. H. Gregory and J. L. Monteith, editors, "Airborne Microbes," University Press, Cambridge, England, 1967.

†A. Andersen, "The New Sampler for the Collection, Sizing and Enumeration of Viable Airborne Particles," J. Bacteriol. 76, 471-484 (1958).

different isolates due to multiple impingement at the same loci. The results are presented in Table A19. The most common organism is *Acinetobacter*, and its most common phenon is 4-1.*

Table A19
Identification of Isolates from Stage 4 of the Andersen Samplers

Day	Organism	Day	Organism
35	*Bacillus pulvifaciens*	50	*Acinetobacter* 4-1
39	*Aeromonas* sp	53	*Acinetobacter* 4-1
40	*Acinetobacter* 4-1	54	*Acinetobacter* 4-2
42	*Acinetobacter* 4-1	56	*Acinetobacter* 4-3
43-1	*Acinetobacter* 4-1	57-1	*Acinetobacter* 4-2
43-2	*Aeromonas* sp	57-2	*Micrococci* sp
46	*Enterobacteria* sp	57-3	*Proteus rettgeri*
47	*Acinetobacter* 4-3		

On two occasions in the course of the study the general bacterial population may have contained potentially pathogenic organisms. On day 15 there were 0.6 mannitol-fermenting organism/ft^3 (as revealed on mannitol salt agar). On day 19 the green-pigment-producing organisms on the *Pseudomonas* medium were at a level of 8/ft^3. (These organisms did not appear at any other time on these media during the program.) Definitive identification of the types of organisms was not made; however, the presumption is that these organisms were *Staphylococcus* and *Pseudomonas* respectively.

A3.4.4.3 Discussion

The number of airborne bacteria in the Tektite I habitat was found to be higher than that found in normal environmental air. A range of values from 7.3 to 29.4/ft^3 were found to be normal by Miller et al.† The peak levels in the habitat exceeded the upper value by 3 to 6 times, and the levels frequently exceeded the upper value by 1 to 2 times. The best-fit line of the general microbial population growing on TSA was higher than 30/ft^3 for 26 consecutive days.

The repeated isolation of *Acinetobacter* subcultured at random from the common colony type appearing on stage 4 reveals a high incidence of that organism in the air. It is unfortunate that the random isolation of the most common colony type was not instituted earlier. Consequently it has been impossible to determine whether the group of *Acinetobacter* isolates from the air were present initially or were introduced during the first half of the study.

During the latter half of the program there was an increase in organisms capable of growth on the marine agar. This increase was greater than that found on the trypticase soy agar (general population) and represents the occurrence of a second, different microbial population that required the marine media. However, this does not in itself necessarily define that group as of marine origin.

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*M. J. Thornley, "Properties of Acinetobacter and Related Genera," pp. 19-28 in B. M. Gibbs and D. A. Shapton, editors, "Identification Methods for Microbiologists, Part B," Academic Press, London, 1968.

†R. L. Miller, W. E. Burton, and R. W. Spore, "Aerosols Produced by Dental Instrumentation," pp. 97-120 in "First International Symposium on Aerobiology," Naval Biological Laboratory, Naval Supply Center, Oakland, California, 1963.

Some of the organisms growing on the TSA may also have grown on the marine agar. The *Aeromonas* isolates may well be from the marine environment, as some *Aeromonas* species have been found to be fish pathogens.* A number of genera were studied by Thornley† who proposed a provisional genus for a group of similar organisms including a number of *Achromobacter* species. The number of *Achromobacter* in sea water was found to be 26% of the cultures examined by Wood.‡ On the surface of fish it has been shown to vary from 53.7% of cultures examined from salmon§ to 23% on haddock.¶ Thus, the incidence of *Achromobacter*, which are partially included in the group *Acinetobacter*, has been shown to be quite common in the marine environment.

The high incidence of *Acinetobacter* on the air samples and the increase in numbers of organisms growing on the marine medium may indicate an intrusion into the habitat by an organism of marine origin.

The demonstration of the mannitol-fermenting organisms in the air on day 15 does not correlate with any entries in the medical log during that period. However, the presence in air of organisms presumed to be *Pseudomonas*, at a level of 8/ft^3 on day 19, is followed on the next day by complaints of ear infections in three divers. Three alternatives seem evident: the *Pseudomonas* in the air may have originated from the infected ears, the ears may have been infected by the organism from the air as a result of its aerosolization from another source, or the infected ears and aerosol *Pseudomonas* may be unrelated. Because of the low frequency of air sampling the particular alternative could not be determined.

The actual numbers of bacteria per cubic foot may have been slightly higher than those expressed. The mean relative humidity was between 50 and 55%, a moisture level generally most detrimental for vegetative airborne bacteria.♦ The resulting growth from the air sampled included only those that survived or were able to recover from the shock of humidity exposure. The bacteria in the smaller particle sizes are more sensitive, again resulting in reduced counts.

A3.4.4.4 Conclusion

The level of airborne bacteria in the habitat was above normal by day 24 of the program. This level stayed elevated for the following 26 days. On two occasions potentially pathogenic organisms may have been present in the air: on day 15 there were 0.6 mannitol-fermenting organism/ft (*Staphylococci*), and on day 19 *Pseudomonas*-like organisms were present at a level of 8 organisms/ft^3.

Acinetobacter phenon 4-1 was the most common organism occurring on stage 4 of the Andersen sampler from day 35 to the completion of the program. This organism may have had its origin in the marine environment, establishing itself on the aquanauts or in the habitat during the latter half of the program.

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*I. W. Smith, "The Classification of Bacterium salmonicida," J. Gen. Microbiol. 33, 263-274 (1963).

†Op. cit.

‡E. J. F. Wood, "Studies on the Marketing of Fresh Fish in Eastern Australia, Part 2 - The Bacteriology of Spoilage of Marine Fish," Australia Council Sci. Ind. Res., Melbourne, Pamphlet 100, 1-92, 1940.

§J. E. Snow and P. J. Beard, "Studies on Bacterial Flora of North Pacific Salmon," Food Res. 4, 563-585 (1939).

¶G. B. Reed and C. M. Spence, "The Intestinal and Slime Flora of the Haddock, A Preliminary Report," Contr. Canad. Biol. Fish., N.S., 4, 257-264 (1929).

♦F. W. Dunklin and T. T. Puck, "The Lethal Effect of Relative Humidity on Airborne Bacteria," J. Exptl. Med. 87, 87-101 (1948).

A3.4.5 Bacteriology

D. N. Wright and Andre B. Cobet,
Naval Biological Laboratory

A3.4.5.1 Introduction

The health and welfare of the aquanauts was of prime importance in achieving the desired performance and effort in the underwater program. The microbial flora associated with the aquanaut can, under adverse circumstances, impair the performance of the divers to the point that they must be removed from the program. Hence it was necessary to study the microflora of the aquanauts and determine the effects of the environmental conditions in the submerged habitat on the aquanaut/bacteria relationship.

An extensive study was made of the type and numbers of bacteria present at five body areas of the four aquanauts and on the walls of two compartments of the habitat. The frequency of occurrence of the various bacteria at the start, during the 59-day program, and at the termination of the underwater period was determined.

A3.4.5.2 Procedure

The samples were received at the laboratory under dry ice and were maintained at -70°C until the time of analysis. Prior to culture the sample was thawed at room temperature and the swab expressed into the vial. The contents of the vials were taken as a 10^{-3} dilution. Tenfold dilutions were made of the contents, with a range of dilutions plated on the surface of selected media; the type of media was determined by the source of the sample, as shown in Table A20. All media were procured commercially (Difco) except blood agar, which was prepared locally with 5% defibrinated sheep red blood cells.

Table A20
Media Used in the Microbial Analysis of the Bacterial Samples

Site	Medium			
	EMB	Blood Agar	Mitis Salivarius Agar	Mannitol Salt Agar
Ear	X	X		X
Forearm skin	X	X	X	X
Skin behind knee	X	X	X	X
Throat	X	X	X	
Rectum	X	X	X	X
Habitat	X	X		X

The inoculated media were incubated aerobically at 37°C for 30 hours. No effort was made to determine the anaerobic or microaerophilic flora. Each bacterial colony type growing on the media was enumerated and subcultured for identification.

Identification of the organisms found on primary isolation was by standard bacteriological procedures, based on selected growth requirements, biochemical reactions, and

morphological characteristics as outlined in the "Manual of Microbiological Methods"* and "Bergey's Manual of Determinative Bacteriology".† No attempt was made to identify all organisms at the species level. When species were indicated as a result of differential procedures, they were recorded. The organisms listed as *Acinetobacter* were gram-negative, nonmotile, largely nonfermentative coccobacilli.‡ Organisms reported as *Staphylococcus albus* included all mannitol-negative, gram-positive cocci with the exception of *Streptococcus* and *Sarcina*. The determination of *Staphylococcus aureus* was made on the basis of mannitol fermentation. No attempt was made to determine the classification of the few yeast and fungal isolates, as a separate section of this report covers their identification.

A3.4.5.3 Results

The scope of the work can be appreciated by observing a few figures: over 500 samples were taken from the body areas and the environment, which resulted in over 2500 different primary cultures to be identified, which in turn required over 4000 plates and tubes of media for final identification.

The method of obtaining the samples by swabbing of surfaces on different occasions places certain restrictions on the direct comparison of the data. Since the total area swabbed in one instance may not equal the area swabbed at another time, the resulting quantitative populations will differ even though qualitatively they may have been the same. Consequently some of the data have been assigned a numerical value based on the quantitative standing of the microbe in relation to others from that same sample rather than as an absolute value.

The recovery of bacteria from the rectal samples of the aquanauts is presented in Table A21. It is apparent that there were no unexpected organisms recovered from these specimens. No bacteria were recovered during the latter portion of the dive which were not also seen in the early phases of the study. Common bowel organisms such as *Escherichia coli*, *Staphylococci*, and *Streptococci* were found consistently during the entire program. *Proteus vulgaris* was recovered from aquanaut 1 periodically through the study but not from other divers. *Aerobacter aerogenes* was recovered during only the early portion of the dive. Whether this organism was lost completely or simply not recovered is unknown.

The recovery of bacteria from the ears during the first third of the study was very good. Antibiotic treatment was given to the aquanauts for external ear infections throughout the latter two thirds of the dive; consequently many samples yielded no growth (Table A22).

Throughout the study *Corynebacterium* and *Staphylococcal* species were consistently isolated from all ears sampled. Aquanaut 4 may have entered the program with *Pseudomonas aeruginosa* as part of the ear flora. The *Pseudomonas* persisted until the antibiotic therapy was instituted for ear infection. It was found again on day 33, followed by a second ear infection in that ear (Table A23). *Pseudomonas aeruginosa* was isolated once from the ears of aquanaut 1, occurring between two episodes of ear infection. *Proteus* was isolated from the ears of aquanaut 1 during periods of ear infection and before antibacterial therapy. *Corynebacterium* was found only sporadically in the ear of

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*Manual of Microbiological Methods," Soc. of Amer. Bact., McGraw-Hill, New York, 1957.
†R. S. Breed, E. G. D. Murray, and N. R. Smith, editors, "Bergey's Manual of Determinative Bacteriology," 7th edition, Williams and Wilkins, Baltimore, 1957.
‡M. J. Thornley, "Properties of Acinetobacter and Related Genera," pp. 29-50 in B. M. Gibbs and D. A. Shapton, editors, "Identification Methods for Microbiologists," Academic Press, New York, 1968.

Table A21
Bacterial Flora in the Rectal Samples Collected From the Tektite I Aquanauts

Day of Dive	Occurrence of Organism in Aquanaut 1, 2, 3, or 4																																			
	Coryne-bacterium				*E. coli*				*S. albus*				*S. fecalis*				*S. salivarius*				*A. aerogenes*				*S. mitis*				*Bacillus*				*P. vulgaris*				*P. aeruginosa*			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0	X	X		X	X	X	X	X		X		X	X		X	X		X		X			X	X													X			
5	X	X	X	X	X	X	X	X	X	X		X	X	X		X	X			X																				
8	X		X	X			X		X	X	X	X		X	X		X																				X			
12		X	X	X		X	X	X		X	X	X		X	X	X				X																				
15	X	X		X	X	X	X	X	X	X	X	X		X	X	X	X						X	X						X										
19	X	X	X			X	X	X	X	X	X	X			X	X		X		X				X																
22	X	X	X	X	X	X		X	X	X	X	X		X	X		X	X		X								X												
26	X	X			X	X		X	X	X	X	X	X	X			X	X		X			X	X		X														
29		X		X		X		X				X	X	X	X		X			X	X					X					X									
33		X	X					X		X	X	X		X		X				X				X														X			
36		X	X					X		X	X			X				X		X																				
40	X	X	X	X		X	X	X		X		X						X		X															X					
43						X		X		X		X																							X					
47			X	X	X			X			X		X	X	X	X	X								X										X		X			
50		X	X	X	X		X				X	X		X	X	X	X	X											X			X								
54	X	X	X		X		X			X		X	X	X			X	X	X						X				X			X								
57			X	X	X		X			X			X				X	X	X	X						X				X										
60			X		X		X		X	X	X	X	X	X	X	X	X	X	X	X																				

Table A22
Bacterial Flora in the Ear Samples Collected From the Tektite I Aquanauts

Aquanaut	Organism	Quantitative Ranking of Occurrence of Organism For Each Aquanaut For Each Day of Dive a Sample Was Taken																
		0	5	8	12	15	19	22	26	29	33	36	40	43	47	50	54	57	60
1	*S. albus*		1	1	1	1	1	1										2	1
	Corynebacterium		3	2	2	2	2	2	1									1	
	Pseudomonas							3											
	E. coli			3															
	Mima		2		3														
	Proteus				4	4	4												
	Neisseria				5	3													
	Aerobacter						3												
2	*S. albus*	2	1	2	1	1	2	1	1	1	1				2				
	Corynebacterium	1		1		2	1	2	2										
	S. aureus						3	3		2									
	S. lutea			3															
	Bacillus									3									
	Acinetobacter			4															
3	*S. albus*	1	1	2	1	1	1	1	1						1	1	1		1
	Corynebacterium		2			2													
	Pseudomonas					4	2	2											
	S. aureus			1															
	E. coli			4	2														
	Proteus				4														
	Aerobacter					3		3											
	Acinetobacter			3	3														
4	*S. albus*	2	1	1	2	2	1	1			3		1				1	1	
	Corynebacterium	1			1	1	3				2		2						
	Pseudomonas		2	2	3			3			1								
	S. aureus						2	2									2		
	E. coli				4														
	Mima					4													
	S. lutea					3													1
	Bacillus															1			

aquanaut 3. *Pseudomonas aeruginosa* was isolated just prior to symptoms of ear infection and before antibiotic therapy as in aquanaut 4. *Corynebacterium* and *Staphylococci* were regularly isolated from aquanaut 2. No gram-negative organisms were isolated from his ear with the exception of a single isolation of *Acinetobacter*. However, this aquanaut also experienced ear infection, even though no etiological organisms were apparent.

There were no unexpected isolations from the throats of the aquanauts, with *Streptococci*, *Corynebacteria*, and *Neisseria* being consistently recovered. *Diplococcus pneumoniae* was isolated from aquanaut 4 during only the first half of the dive (Table A24).

Table A23
Medical Status of Aquanauts' Ears During the Tektite I Program
(Data Obtained From the Medical Status Reports in the Medical Log)

Day of Dive	Status of Ear							
	Aquanaut 1		Aquanaut 2		Aquanaut 3		Aquanaut 4	
	Right	Left	Right	Left	Right	Left	Right	Left
2	-	-	-	-	-	-	Questionable	-
3	-	-	-	-	-	-	Questionable	-
7	-	-	-	-	-	-	Surge effect	
9	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-
12	-	Squeeze	-	Squeeze	-	-	-	-
14	-	-	-	-	-	Squeeze	-	-
15	-	-	-	-	-	-	-	-
16	-	Squeeze	Squeeze	-	-	-	-	-
18	Squeeze	-	-	-	-	-	-	-
20	Infected	-	Squeeze	-	Infected	-	Infected	-
22	-	-	Squeeze	-	Infected	-	Infected	Infected
25	-	-	-	-	Healing	-	Infected	-
27	Infected	-	Infected	-	Healing	-	Infected	-
29	Infected	-	-	-	-	Squeeze	-	Redness
31	Infected	Infected	-	-	-	-	-	-
33	-	-	-	-	-	-	-	Infected
34	Infected	-	-	-	-	-	-	Infected
36	Infected	Infected	Swollen	-	-	Squeeze	Infected	Infected
37	-	Infected	Redness	-	-	-	Infected	Infected
38	-	Infected	Infected	-	-	-	Infected	Infected
39	-	Infected	Infected	-	-	-	Redness	Redness
40	-	Swollen	Swollen	-	-	-	Redness	-
41	-	-	-	-	-	-	-	-
42	-	-	-	-	-	-	-	-
44	-	-	-	-	-	-	-	-
46	-	-	-	-	-	-	-	-
49	-	-	-	-	-	-	-	-
52	-	-	-	-	-	-	-	-
53	-	-	-	-	-	-	Squeeze	-
54	-	-	-	-	-	-	-	-
55	-	-	-	-	-	-	-	-
57	-	Squeeze	Squeeze	-	-	-	Squeeze	Squeeze
58	-	Squeeze	Squeeze	-	-	-	Squeeze	Squeeze

The greatest variety of microbial flora was found on the skin of the forearm and behind the knee. The bacteria from these sample sites was similar, both in types and numbers with the routine isolation of *Staphylococcus*, *Corynebacterium*, and some *Streptococcus* (Tables A25 and A26). On occasion a number of organisms from the genera *Bacillus*, *Mima*, *Aerobacter*, and *Escherichia* were isolated and may represent a transient population associated with the skin. A third population was evident on the skin of aquanauts 1, 2, and 4. This group, consisting of members from the genera *Sarcina* and *Acinetobacter* were not found until the latter phase of the study. In contrast, these organisms were isolated with regularity during the entire study from the knee of aquanaut 3. These two groups of organisms may represent a progressive change in the skin flora of

Table A24
Bacterial Flora in the Throat Samples Collected From the Tektite I Aquanauts

Day of Dive	Occurrence of Organism in Aquanaut 1, 2, 3, or 4																																										
	S. salivarius				*S. mitis*				*Neisseria*				*Coryne-bacterium*				*S. albus*				*S. fecalis*				*Pseudo-monas*				*Acineto-bacter*				*Bacillus*				*S. aureus*				*D. pneu-moniae*				*P. vulgaris*			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0		X		X	X		X	X					X	X			X	X	X				X	X				X													X							
5	X	X	X	X		X	X		X	X			X	X						X																						X						
8	X		X	X	X		X	X	X			X				X	X		X					X																	X						
12	X	X	X	X	X	X	X	X	X					X						X																				X		X				
15	X	X	X	X		X		X		X				X	X	X							X																		X		X				
19	X	X	X	X	X	X	X	X	X	X	X	X				X																									X					
22	X	X	X	X	X	X	X	X	X		X			X					X																					X					
26	X	X	X	X	X	X	X	X	X	X			X		X	X																X				X				X					
29	X	X	X	X	X	X	X	X	X	X		X			X						X																								
33	X	X	X	X	X	X	X	X	X	X		X																				X								X					
36	X	X	X	X	X	X	X	X	X	X		X	X			X		X		X																			X						
40	X	X	X	X	X	X	X	X	X	X		X								X																									
43	X	X	X	X		X	X	X	X	X		X	X			X			X																										
47	X	X	X	X		X		X	X	X	X	X			X		X	X		X																									
50	X	X	X	X	X	X		X		X		X	X			X		X		X																						X			
54	X	X	X	X	X	X		X	X	X		X	X		X	X	X			X																									
57		X	X	X		X		X				X																																	
59		X		X		X		X	X				X				X	X		X																									

Table A25
Bacterial Flora of the Skin (Forearm) Samples Collected From the Tektite I Aquanauts

Day of Dive	Occurrence of Organism in Aquanaut 1, 2, 3, or 4																																										
	Coryne-bacterium				*S. albus*				*S. aureus*				*S. salivarius*				*E. coli*				*S. fecalis*				*Bacillus*				*Mima*				*Aero-bacter*				*S. mitis*				*S. lutea*				*Acineto-bacter*			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0	X	X			X	X	X	X		X	X														X	X	X	X		X												X							
5		X	X	X		X	X	X		X		X		X				X																													
8	X		X	X	X	X	X	X				X														X														X								
12	X	X	X	X	X	X	X	X														X		X						X									X							X
15	X	X	X	X	X	X	X	X	X	X		X		X		X						X				X	X		X				X							X							
19		X	X	X	X	X	X	X	X	X	X	X		X		X						X		X		X	X								X						X						
22	X	X	X	X	X	X	X	X	X	X		X				X										X			X								X				X	X				X	
26	X	X	X	X	X	X	X	X	X	X		X		X			X											X				X														X
29	X	X	X	X	X	X	X	X	X	X	X	X									X					X				X											X	X	X				X
33	X	X	X	X	X	X	X	X	X	X				X								X	X			X				X									X		X	X		X	X		X
36		X		X		X		X						X																														X			
40	X	X		X	X	X	X	X	X					X																												X	X	X		X		X
43		X	X		X	X	X																				X				X	X								X			X	X	X		X
47			X	X	X	X	X	X		X												X									X									X			X		X		
50	X			X	X		X	X														X									X									X				X		X
54	X	X	X		X	X	X	X														X						X												X	X			X	X		
57	X	X	X	X	X	X	X	X																	X																X	X		X	X		X
60	X							X	X		X																														X	X	X	X	X	X		X

Table A26

Bacterial Flora of the Skin (Behind the Knee) Samples Collected From the Tektite I Aquanauts

Day of Dive	Occurrence of Organism in Aquanaut 1, 2, 3, or 4																																							
	Coryne-bacterium				*S. albus*				*S. aureus*				*S. salivarius*				*E. coli*				*S. fecalis*				*Bacillus*				*Mima*				*Aero-bacter*				*P. vulgaris*				*S. mitis*				*S. lutea*				*Acineto-bacter*			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
0	X				X	X	X	X	X	X		X						X					X		X		X		X																				
5					X	X	X	X	X													X	X																										
8	X	X	X	X	X	X	X	X	X	X		X										X	X			X																						
12	X	X	X	X	X	X	X	X	X			X				X													X														X	X					
15	X	X	X		X	X	X		X	X	X							X				X																											
19	X	X	X	X	X	X	X	X	X	X		X		X		X				X				X						X												X	X					
22		X	X	X		X	X	X	X	X		X		X								X		X		X		X															X					
26	X	X	X	X	X	X	X	X	X	X		X						X				X		X					X													X						
29	X	X		X	X	X	X	X	X	X		X		X				X	X							X																X	X					
33	X				X		X		X	X												X																										
36	X	X		X		X		X	X	X		X												X		X																X	X					
40	X	X	X	X	X	X	X	X	X			X						X												X	X										X	X					
43		X	X		X	X	X	X																		X																					
47	X	X		X	X	X	X	X	X	X						X								X																							
50	X	X	X	X	X	X	X	X				X		X								X				X				X																
54	X	X		X	X	X	X	X		X								X																									X	X					
57	X	X	X		X	X	X	X																																			X		X	X			
60	X				X	X		X		X																																	X	X	X	X			

the aquanauts. A second change in skin flora is suggested by failure to recover mannitol-fermenting *Staphylococci* during the last half of the study. The relationship, if any, between the loss of this organism and the appearance of *Acinetobacter* and *Sarcina* is not known.

The samples collected from the skin sites (forearm and behind the knee) before entry of the aquanauts into the habitat yielded no *Acinetobacter* isolates (Table A27). As the study progressed, the frequency of isolation increased, ultimately involving all four aquanauts and the habitat.

Table A27
Frequency of Isolation of Acinetobacter From the Two Skin Sites of the Aquanauts and in the Habitat

Day of Dive	Occurrence of *Acinetobacter* Isolates									
	Aquanaut 1		Aquanaut 2		Aquanaut 3		Aquanaut 4		Habitat	
	Arm	Knee	Arm	Knee	Arm	Knee	Arm	Knee	Wet Lab	Crew Quarters
0										
5										
8			X			X				
12						X				
15					X	X			X	
19		X								
22			X		X		X			
29	X	X	X				X			
33	X						X	X		
36			X							
40		X	X		X					
43	X	X	X			X	X	X		
47			X					X		
50						X	X	X	X	X
54	X	X	X					X		
57	X	X	X			X	X	X		
59	X	X	X			X	X	X	X	X

The recovery of organisms from the wall surfaces of the wet lab was irregular. In the crew quarters microbial flora was more apparent, as judged by the greater frequency of isolation. In both rooms the predominant genera were *Staphylococcus* and *Bacillus* (Table A28).

A3.4.5.4 Discussion

The aquanauts were active in two entirely different environments during the course of the Tektite I program. They each had marine science programs requiring their presence in the wet marine environment as well as in-habitat chores required by both the marine science program and their daily living. The two environments and the introduction of new or reintroduction of familiar organisms with food and equipment by way of the daily transfers from the surface and the continuous input of air via the umbilical from the surface removes the study from the isolated-environment group. Thus it is not surprising that a unity in the type of flora of the divers did not occur. It was not expected, however, that normal flora would be isolated in the latter phase of the study which were not present in the early samples.

Table A28
Bacterial Flora in the Samples Collected From the Two Spaces in the Tektite I Habitat

Day of Dive	Population (10^4 organisms)												
	Wet Lab							Crew Quarters					
	S. albus	*Bacillus*	*Coryne-bacterium*	*S. lutea*	*Mima*	*S. mitis*	*Acineto-bacter*	*S. albus*	*Bacillus*	*Coryne-bacterium*	*S. lutea*	*Mima*	*Acineto-bacter*
0	-	-	-	-	-	-	-	3.0	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	1.0	1.0	-	-	-	-
12	1.0	-	-	-	-	-	-	1.0	1.0	-	-	-	-
15	-	1.0	-	-	-	-	2.0	2.0	11	-	-	-	-
19	-	-	-	-	-	-	-	1.0	16	-	-	-	-
22	1	-	-	-	-	-	-	3.0	-	1.0	-	-	-
26	-	190	-	-	-	-	-	-	0.1	-	-	-	-
29	-	-	-	-	-	-	-	-	-	-	-	-	-
33	-	-	8.0	-	-	-	-	-	-	-	-	-	-
36	-	-	-	-	-	-	-	2.0	-	-	-	-	-
40	3.0	-	-	-	-	-	-	1.0	2.0	-	-	2.0	-
43	-	-	-	-	-	-	-	2.0	-	-	-	-	-
47	-	-	-	-	-	-	-	2.0	-	-	-	-	-
50	-	-	-	-	2.0	8.0	1.0	6.0	-	10	1.0	1.0	1.0
54	-	4.0	-	-	-	-	-	2.0	-	-	-	-	-
57	-	-	-	-	-	-	-	-	-	-	-	-	-
59	-	-	-	1.0	-	-	4.0	-	-	-	-	-	1.0

In several instances some organisms were not found in each sample of a series. It is possible that they were present but not recovered from the sample, although this is unlikely, since the isolation procedures were adequate throughout most of the work. Another explanation may be the existence of a cycle with a succession of organisms, as has been demonstrated in the intestine.* There may have been periods during which the number of a particular organism was low and was not recovered by this technique; alternately it may have been completely eliminated and reappeared only after reinfection. This phenomenon would explain the disappearance of *A. aerogenes* from the rectal samples and may also shed light upon the disappearance of mannitol-fermenting *Staphylococci* from the skin.

The transmission of bacteria between the men in the habitat will be presented in section A3.4.9 (on nasal *Staphylococcus*). Tracer organisms were not introduced into the habitat, nor were the organisms recovered in this study phage or serotyped. However, there were several isolates of *P. aeruginosa* from the ears of three aquanauts, although it appears that this organism was present at the outset in only one aquanaut. Whether the subsequent appearance of this organism from the ears, skin, and feces or the other aquanauts represents man-to-man as opposed to environment-to-man transmission is unknown.

There was one organism which occurred consistently in one aquanaut only, that being *Proteus*, which was isolated from rectal samples from aquanaut 1. This organism was apparently not transmitted to the other aquanauts, even though all were in intimate contact. This supports the concepts that each person has his own bacterial profile and that the establishment of a new organism largely depends on the organisms already present within that environment.

An infection of the aquanauts by organisms from the ocean environment did not occur. The organisms of the genus *Acinetobacter* are common in terrestial and water environments,† and their establishment on the skin of the aquanauts appears to have been a commensal association. Had the aquanauts been subjected to mechanical injury or other stress factors, it is possible that these organisms may have become involved in a pathogenic situation. *Staphylococcus lutea* increased as part of the skin flora; although normally found as part of this flora, it is of interest primarily because of its emerging dominance. What possible health involvement could result from the continued high numbers of these two organisms is as yet to be learned.

One of the most interesting findings is concerned with the isolation of *P. aeruginosa* from the ears of the aquanauts. External ear infections are of particular concern among men who do a great deal of marine diving or swimming. These ear infections respond to antibiotic therapy with no unusual sequelae and are commonly thought to be due to *P. aeruginosa*. This organism may have been responsible for the external ear infections suffered by aquanauts 3 and 4. It is unlikely, however, that the ear infections of aquanauts 1 and 2 resulted from such an infection. *Proteus*, which was isolated from both rectum and ears of aquanaut 1 may have been responsible for the infection in his ears, but aquanaut 2 at no time demonstrated any unusual flora which would suggest the etiology of ear infection. It is perhaps significant that this aquanaut had two ear infections and that the longest persisted for only 4 days. An explanation as to the cause of such ear infections is not available. Ear samples were obtained from swabs of the right ear only. In aquanauts 1 and 4, the right ear was initially infected, and in aquanauts 2 and 3 only the right ears were involved. It is possible that swabbing improved the opportunity for infection, although it was not the sole predisposing factor.

*R. J. Dubos and J. G. Hirsch, editors, "Bacterial and Mycotic Infections of Man," 4th edition, Lippincott, Philadelphia, 1958.

†M. Ingram and J. M. Shewan, "Introductory Reflections on the Pseudomonas-Achromobacter Group," J. Appl. Bacteriol. 23(3), 373-378 (1960); M. J. Thornley, "A Taxonomic Study of Acinetobacter and Related Genera," J. Gen. Microbiol. 49, 211-257 (1967).

No attempt was made to restrict medication to the divers during the program. While the aquanauts suffered from these ear infections, they were restricted from diving and were given a therapeutic regimen of cortisporin ear drops. On day 38 all aquanauts were started on colymycin ear drops and oral achromycin. The achromycin therapy was discontinued on day 43, but the colymycin was used throughout the remainder of the dive.

This study suggests that no saprophytic species of bacteria were present in the Tektite I environment which became pathogenic or which were predisposed to enhanced virulence as a result of the environmental conditions surrounding the aquanauts. Other studies, however, have shown numerous situations where changes in the normal flora result in disease.* The ultimate effect of long-term changes of skin flora as noted above are of course not known and subject to understanding only by prolonging such an experimental condition.

The use of systemic antibiotics may have resulted in possible alteration of oral and intestinal flora. It is suggested that in future situations internal antibiotic therapy be reserved until other procedures were deemed ineffective. Of the external medications, colymycin appeared to be most effective in reducing the otitis externa. However, this antibiotic was used concomitantly with an alcohol-boric acid wash of the ear, so that a true evaluation of effectiveness was not possible.

A3.4.5.6 Conclusions

A number of conclusions can be drawn from the data obtained during this study. The most obvious result suggested by the data is that man can exist, live, and work under conditions of this experiment relatively free from microbial hazards. The fact that there was little or no change in the microbial flora of the oral cavity and intestinal tract suggests that the imposed external environment had little or no effect on these body areas in terms of their ability to support microbial life.

The study also suggested that those areas of the body with the greatest exposure to the environment were most readily affected in terms of their microbial flora. The increase in the number and frequency of isolation of *Acinetobacter* is evidence of change in the normal flora of these areas, and this buildup in the habitat and on the skin represents a significant alteration in the environmental microflora of unknown consequence. The question as to whether or not this condition represents a hazard to men in this environment has not been answered.

The ear infections from microbial flora of the external ear canal represent the only recorded incidences of microbial illness during the dive. However, these infections were not significantly different from those ear infections seen in divers who were operating under less severe environmental circumstances. Indeed, in view of the frequency of this disease among divers, the occurrence of some otitis was to be expected.

A3.4.5.7 Acknowledgments

The authors acknowledge the assistance of Lt. Phyllis Warren, and HM3 Charles Williston.

*R. J. Dubos and J. G. Hirsch, editors, "Bacterial and Mycotic Infections of Man," 4th edition, Lippincott, Philadelphia, 1958.

A3.4.6 Mycology

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A3.4.6.1 Introduction

The microflora associated with man, and his environment, plays an important role in his well being. This association becomes quite important when man is restricted to an environment of an unusual nature for an extended period. The mycological aspects of the microflora were studied during the 59-day program.

It was not the intent of the survey to determine quantitatively or qualitatively the total fungal and yeast flora of certain sites on aquanauts and their environment but rather to ascertain the predominant genera or types and their relative numbers and changes during the program. In particular the early detection of dermatophytes was sought if skin infections proved to be a problem.

A3.4.6.2 Procedures

The mycology media were prepared and inoculated at the base camp and shipped by air to the Naval Biological Laboratory, where total numbers of fungi and yeasts were determined on arrival and after incubation at 37°C for 8, 14, 21, and 42 days. In most cases each morphologically distinguishable colony type was isolated and characterized generically in the case of fungi or with reference to tribe or section in the case of yeasts. The systematic key of Wilson and Plunkett* was employed largely for fungal taxonomy, but use was made also of criteria outlined by Skinner, Emmons, and Tsuchiya† and in the National Communicable Diseases Center Manual.‡ The classification of yeasts followed that described by Henrici.§

Predive samples were taken from some of the aquanauts 38 days before the dive and from all of them on the morning of the dive (day 0) shortly before entering the water. The total numbers of fungi and yeast from all media are reported. It was believed that this procedure provided the best available approximation of relative numbers. In those instances where one of the three media showed too many colonies to be counted, a value of 100 was assigned; to distinguish that the 100 was an approximate value the graph point representing it was drawn with an arrow through it. Where two or more plates were uncountable, a value of 200 was assigned and the same symbol was used. The letters and numbers alongside each point show the numbers of each category of fungus and/or yeast (by code) represented by the point.

A3.4.6.3 Results and Discussion

The mycofloral pattern of the aquanauts and of the walls in the wet lab and crew quarters of the Tektite I habitat are presented in Table A29 and Figs. A34 through A39. Table A30 shows the frequency with which the 53 fungal or yeast varieties identified during the study were recovered. It should be emphasized that changes in the varieties and

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*J. W. Wilson and O. A. Plunkett, "The Fungus Diseases of Man," Univ. of Calif. Press, Berkeley, 1965.

†C. E. Skinner, C. W. Emmons, and H. M. Tsuchiya, "Henrici's Molds, Yeasts and Actinomycetes," Wiley, New York, 1948.

‡L. Ajello, L. K. Georg, W. Kaplan, and L. Kaufman, "Laboratory Manual for Medical Mycology," U.S. Dept. of Health, Education, and Welfare, Atlanta, Public Health Service Publication 994, 1963.

§A. T. Henrici, "The Yeasts: Genetics, Cytology, Variation, Classification and Identification," Bact. Rev. 5, 97-179 (1941).