

Study of Man During a 56-day Exposure to an Oxygen-Helium Atmosphere at 258 mm. Hg Total Pressure

II. Major and Minor Atmospheric Components

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The atmosphere to which four human volunteers were exposed for 56 days during a study designed to describe the effects of oxygen-helium on man was analyzed for major and minor constituents. The partial pressure of the major constituents, oxygen (175.2 ± 2.4 mm. Hg) and helium (73.9 ± 2.3 mm. Hg), remained within the established experimental parameters. Sixty-eight minor constituents were detected. The concentration of these compounds remained below a level thought to cause a physiologic effect. The instrumental methods employed were sufficient for a comprehensive analysis of the synthetic atmosphere.

IN THE DETERMINATION of the physiologic suitability of potential spacecraft atmospheres, detailed knowledge of both the major and minor or "trace" con-

stituents of the atmosphere is a vital portion of any such study. Detailed knowledge of the major gaseous constituents is essential in order to insure that the desired gases are present and present in the appropriate concentrations. Equally as important is the requirement for knowledge of the trace constituent concentrations, since abnormal levels of these gases could lead to aberrant physiologic results and complicate the study of the atmosphere under question.

The purpose of this paper is to describe the atmospheric composition portion of an experimental program studying the effects of an oxygen-helium atmosphere on man.

METHODS

The test cell gas analysis system, described in the previous paper,³ has been successfully used for atmos-

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pheres composed of oxygen, nitrogen, and carbon dioxide. However, helium in the sample stream was found to affect the analyzers when they were calibrated using nitrogen and/or oxygen as the background gas. Methods to compensate for this effect were successful, with the exception of the nitrogen analyzer in which attempts to "zero out" the helium effect were unsuccessful. Therefore, this analyzer was used only during the ground-level phase of the experiment, 80 per cent nitrogen-20 per cent oxygen.

Despite the inability to accurately measure the nitrogen concentration with the "Nitralyzer," it was, nevertheless, essential for the successful evaluation of the atmosphere to determine the nitrogen concentration in addition to that of helium and oxygen. This was accomplished gas chromatographically. The system designed was required to separate and detect small quantities of nitrogen, high concentrations of oxygen, and at the same time, produce a quantitative measurement of helium. The chromatograph used contained a 6' x 1/4" column packed with 5 Å molecular sieve, 60-80 mesh size. This packing material was activated for 6 hours at a temperature of 350°C., cooled, and immediately packed. This column was preceded by a 3-foot drier section containing 30-60 mesh silica gel. The oven was operated at 30° C. along with an argon carrier gas flow rate of 20 cc./min. These parameters yielded retention times of 2.0 min for helium, 6.0 min for oxygen, and 10.5 min for nitrogen.

Samples were presented to the chromatograph by a diaphragm pump located in the test cell. A pressure relief valve, set at a pressure of 800 mm. Hg, was placed in the sample line between the pump and the test cell penetration. This allowed the excess sample from the pump to remain in the test cell. This configuration insured a positive pressure, relative to ambient pressure (760 mm. Hg), in the sample manifold located adjacent to the test cell. The sample manifold, connected to the chromatograph sample valve, contained a 0-800 mm. Hg pressure gauge, a water vapor partial pressure transducer, necessary connections for the calibration gases, and sampling ports for the other analyzers, i.e., mass spectrometer, infrared spectrometer, etc. The water vapor partial pressure transducer provided the capability to ascertain the water concentration in the standards as well as the sample. This point was essential for a valid calculation of the helium, oxygen, and nitrogen concentration on a dry gas basis.

It was necessary to obtain the dry gas concentration since the test cell sample, containing a relative humidity of 30-40 per cent, was compressed from 258 to 800 mm. Hg, on occasions condensing water in the lines.

Sampling pressure for the chromatograph was 760 mm. Hg. Calibration was performed on a daily basis, utilizing a percentage bracketing technique. The lower detectable limit for nitrogen, using a 5cc. sample loop, was 0.05 per cent.

All supply gases were analyzed with the chromatograph and with a time-of-flight mass spectrometer. The spectrometer was operated and calibrated using the conventional batch mode and a partial pressure ratio relationship for calculation rather than the conventional

partial pressure sensitivity method. Better precision and accuracy were gained by this technique. In addition to the supply gas analysis, the mass spectrometer was used as a backup system and as a periodic check of the other analyzers.

Considering the duration of this study, it was mandatory that the trace contaminants be monitored to insure the health and welfare of the subjects. Two approaches to the trace gas analysis were used, direct sampling techniques and cryogenic concentration and subsequent analysis.

Of particular interest were the concentrations of carbon monoxide and methane. In previous experiments,² rapid buildup of these compounds was noted. Methane was determined with a gas chromatograph, using a flame ionization detector. The column material, 5 Å molecular sieve, 60-80 mesh, was packed into a 12' x 1/4" copper tube, encased in an oven maintained at 100° C. Helium carrier gas was used at a flow rate of 60 cc./min. The lower detectable limit of methane, using a 5 cc. sample loop, was 1 mg/m³.

Determinations of carbon monoxide were made by Mine Safety Appliance's "Lira" infrared analyzer. A confirmation of the concentration was made with a Beckman IR-7 spectrophotometer, employing a 10-meter multipath gas cell. In addition, the IR-7 was used as a screen for high levels of contaminants. A scan of the infrared spectrum from a frequency of 600 cm⁻¹ to 4000 cm⁻¹ was performed daily. Any rapid buildup of compounds that absorb infrared radiation in this region would have been immediately detected using this technique.

Gas chromatography, in addition to the IR-7, was used to scan for trace contaminants. The chromatograph contained dual columns and a dual flame ionization detector. One of the 12' x 1/8" columns was packed with 1,2,3-tris (cyano-ethoxy) propane on a 60-80 mesh Gas Chrom P solid support. The other was packed with poly propylene glycol on 60-80 mesh support. Both columns had a liquid substrate load of 15 per cent. The oven was isothermally operated at 70° C. along with a helium carrier gas flow rate of 30 cc./min.

One of the major problems involved in trace gas analysis is the loss of the contaminant in the process of obtaining a representative sample. Many organic compounds such as medium boiling alcohols, ketones, aromatics, etc., will be condensed upon the compression of the sample from a pressure of 258 to 760 mm. Hg. To minimize this problem, a closed-circuit sampling system operating at 258 mm. Hg was utilized. The sample was drawn from the test cell through a stainless steel line which was connected to the gas chromatograph sample valve containing a 10 cc. loop. The return line terminated at a diaphragm pump located in the test cell. The pump, operating in the vacuum mode, thus produced a flow of sample from the test cell through the sample loop and back. This system allowed the sample to be taken at a pressure which was slightly below that of the chamber, thus preventing condensation due to compression.

The sensitivity of this system was such that an

organic contaminant could be detected at a concentration which was 10 to 100 times below the level thought to cause a physiologic response. Throughout the duration of the experiment, the contaminant concentration did not reach the level of detectability of this system.

Although the contaminant levels were below the detectable limit of the gas chromatograph, it was, nevertheless, important to determine the level of contamination existing in the chamber. To ascertain a quantitative determination of the trace contaminants, a cryogenic system¹ consisting of three trapping cylinders maintained at 0° C, -78° C, and -175° C, respectively, was used to freeze out, trap, and, thus, concentrate the contaminants before analysis. Upon passing the chamber atmosphere through the trapping cylinders, a compound will be concentrated if the vapor pressure at the trap temperature is less than its partial pressure in the sample stream.

A portion of the chamber atmosphere was passed through a closed circuit system by means of a circulation pump at a pressure of 258 mm. Hg and a flow rate of 500 standard cc./min for a period of 10 hours. This produced a concentration of approximately 500 times that in the test cell atmosphere. To minimize oxidation and catalytic conversions, the sample cylinders were packed in dry ice preceding shipment to two independent analytical laboratories. The techniques used to analyze the trapped gases have been discussed elsewhere.²

RESULTS

A summary of the major atmospheric components during the 56-day test is given in Table I. A combination of the data obtained from the different instrumental techniques was made on a dry gas basis and then related to the test cell pressure by the inclusion of the water partial pressure, which was measured by a transducer located in the test cell. The mean partial pressures plus the 56-day deviations are shown along with the method used and the analytical precision.

A rapid increase in the concentrations of carbon monoxide and methane was observed as in a previous study.² The carbon monoxide level within the chamber ranged from a low of 1.0 mg/m³ to a high of 9.5 mg/m³ with a 56-day mean of 6.4 mg/m³. The con-

centration of methane within the chamber had a low of 4.7 mg/m³, a high of 13.7 mg/m³ and a mean of 7.1 mg/m³. It should be noted that this does not represent total production of these two compounds, since the test cell was not operated as a completely sealed unit.

Results of the analysis of the cryogenically concentrated trace contaminants are shown in Table II. Eighteen samples were evaluated during the experiment. The results are given in terms of chemical class, highest concentration observed, and the number of times a compound was found out of the possible 18 sampling periods. The values reported are not production rates and, therefore, can only be used as a guide to the magnitude of concentration.

DISCUSSION

As previously stated, helium was found to cause biasing effects on the simulator's analysis system. The paramagnetic effect of helium upon the oxygen analyzer was compensated for by the inclusion of approximately 30 per cent helium in the standard gases. The "Lira" carbon dioxide and carbon monoxide analyzers, which utilize infrared absorption measured with a thermal detector, are normally standardized using oxygen and/or nitrogen as background gases. A lower output signal was observed when helium was present as compared to its absence. This significant error was eliminated by the inclusion of 30 per cent helium in the background gases. This fact indicates that the noted effect is due primarily to the large difference in the thermal conductivity between helium and that of oxygen or nitrogen. Although helium does not absorb radiation at the wave length used for carbon dioxide and carbon monoxide, the analyzer, being a thermal system, can lose energy by an increase in the thermal conductivity of the gases present.

A somewhat different but significant effect was found when 30 per cent helium was introduced into the "Nitralyzer." A positive but varying bias was noted. The "Nitralyzer" utilizes the emission spectrum of nitrogen for its detection. Considering this principle, the observed bias was probably due to a slight overlap of the helium and nitrogen spectra. This overlap could not be compensated for by the addition of helium in the background gases.

The instrumentation selected for use in this study performed quite satisfactorily with a minimum of maintenance. The summation of the partial pressures, as summarized in Table I, is greater than the mean total pressure as determined by a pressure gauge. Considering the relative analytical precision, the combination of analytical techniques, and the fact that many different individuals performed the analyses, the results show that the desired partial pressures were held to a small deviation.

A large number of trace contaminants were isolated, identified, and quantitated during the 56 days the test cell was maintained at altitude. The sources of these contaminants are at least threefold: the men themselves, the oxygen and helium supply, and the test cell

TABLE I. SUMMARY OF THE ATMOSPHERIC COMPOSITION

	Mean Partial Pressure (mm. Hg)	Method Used	Relative Analytical Precision
Oxygen	175.2 ± 2.4	GC*	± 0.2%
Helium	73.9 ± 2.3	GC	± 0.2%
Nitrogen	1.9 ± 0.57	GC	± 0.6%
Carbon Dioxide	2.4 ± 0.7	"Lira"***	± 0.1%
Argon	0.41 ± 0.11	MS***	± 2.5%
Water Vapor	7.3 ± 0.4	DEWCEL†	± 2.0%
Summation of the Partial Pressures	261.1 ± 2.5		
Total Pressure	257.7 ± 0.6	W.T. Gauge‡	± 0.2%

*GC = Gas Chromatograph

***"Lira" = Infrared Analyzer

***MS = Mass Spectrometer

†DEWCEL = Foxboro Water Vapor Transducer

‡W. T. Gauge = Wallace & Tiernan Pressure Gauge

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TABLE II. TRACE CONTAMINANTS ISOLATED FROM THE TEST CELL ATMOSPHERE DURING THE 56-DAY STUDY

Compound	Number of Times Reported	Highest Concentration mg/m ³ x 10 ⁻³	ACGIH* TLV 100 mg/m ³ x 10 ⁻³
<i>Alcohols</i>			
Allyl Alcohol	4	4.5	50
n-Butyl Alcohol	4	17.0	3000
iso-Butyl Alcohol	2	1.0	***
Ethyl Alcohol	13	46.0	19000
Methyl Alcohol	9	125.0	2600
n-Propyl Alcohol	4	33.0	***
iso-Propyl Alcohol	8	85.0	9800
<i>Aldehydes</i>			
Acetaldehyde	3	2.7	3600
Butyraldehyde	6	35.0	***
<i>Aromatic Hydrocarbons</i>			
Benzene	14	61.0	800
Ethyl Benzene	4	3.5	4350**
Naphthalene	2	9.3	500**
Toluene	16	85.0	7500
Xylene	10	122.0	4350**
<i>Halogen Derivatives of Aromatic Hydrocarbon</i>			
Chlorobenzene	9	29.0	3500
<i>Halogen Derivatives of Ethane</i>			
1,2-Dichloroethane	4	8.6	2000
Freon 113	5	3.3	***
Freon 114	8	11.0	70000
Methyl Chloroform	2	16.0	19000
Pentafluoroethane	2	60.0	***
<i>Halogen Derivatives of Ethylene</i>			
Perchloroethylene	7	18.0	6700
Trichloroethylene	9	14.0	5200
Vinyl Chloride	3	53.0	13000
<i>Halogen Derivatives of Methane</i>			
Carbon Tetrachloride	7	36.0	650
Chloroform	4	41.0	2400
Freon 11	8	210.0	56000
Freon 22	2	5600.0	***
Methylene Chloride	7	36.0	17500
<i>Ketones</i>			
Acetone	14	140.0	24000
2-Methyl Butanone-3	1	3.7	***
Methyl iso-Butyl Ketone	6	27.0	4100
Methyl Ethyl Ketone	12	590.0	5900
<i>Naphthenes</i>			
Cyclohexane	13	35.0	10500
Decalin	7	330.0	***
Dimethyl Cyclohexane	2	2.4	***
Methyl Cyclohexane	4	5.6	20000
<i>Paraffins</i>			
2,2-Dimethyl Butane	6	4.2	***
2,3-Dimethyl Butane	1	0.032	***
Heptane	1	120.0	20000
Hexane	3	81.0	18000
2-Methyl Pentane	1	0.042	***
Octane	2	58.0	23500
Pentane	2	53.0	29500
iso-Pentane	7	8.5	***
<i>Esters</i>			
Amyl Acetate	1	14.0	5250
iso-Butyl Acetate	4	16.0	7000**
Ethyl Acetate	7	15.0	14000
Ethyl Formate	2	0.39	3000
Methyl Acetate	6	4.2	6100
Methyl n-Butyrate	7	3.7	***
Methyl Methacrylate	4	3.8	4100
n-Propyl Acetate	3	15.0	8400
<i>Ethers</i>			
Dimethyl Furan	1	0.84	***
Ethyl Ether	9	24.0	12000
Furan	2	1.2	***
Methyl Furan	3	16.0	***
iso-Propyl Ether	1	24.0	21000
Tetrahydrofuran	4	14.0	5900
<i>Sulfur Compounds</i>			
Dimethyl Sulfide	1	0.47	***
Ethyl Mercaptan	4	35.0	250**

Methyl Mercaptan	1	23.0	200**
Propyl Mercaptan	3	1.3	***
<i>Olefins</i>			
Ethylene	9	19.0	***
Propylene	8	25.0	***
<i>Diolefin</i>			
Isoprene	8	4.4	***
<i>Halogen Derivatives of Higher Aliphatic Hydrocarbons</i>			
Propyl Chloride	1	1.5	***

*Threshold Limit Value for 1965, adopted at 27th Annual Meeting of American Conference of Governmental Industrial Hygienists, Houston, Texas, May 1965.

**Tentative Value ACGIH, 1965.

***Value not established.

and associated equipment. It would appear that these contaminants were well below the level of toxicity and should not have influenced the physiologic results. An arbitrary level of 0.01 of the American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit values⁴ is shown in Table II for comparative purposes. This type of reduction of the ACGIH values, while not documented nor, perhaps, justified, does provide an index for comparison. The highest concentrations measured in the test cell were 100 to 1000 times less than the 0.01 reduction of the ACGIH values, further strengthening the impression that trace contaminants were not a critical or limiting factor in this study.

It is concluded that the ultimate purpose of the 56-day study, i.e., to compare the use of helium as a diluent gas to that of nitrogen, was accomplished. The oxygen partial pressure was maintained at a level adequate to maintain alveolar oxygen partial pressure equivalent to that observed at ground level. The concentration of trace contaminants never exceeded a level thought to cause a measurable physiologic effect. If there had been a physiologic response due to helium, neither the oxygen partial pressure nor the trace contaminants would have interfered with its observation.

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Study of Man During a 56-day Exposure to an Oxygen-Helium Atmosphere at 258 mm. Hg

Total Pressure

III. Renal Response

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To assess the effects of helium on renal function, four healthy Air Force officers lived for 56 days in a space cabin simulator with a partial pressure of oxygen of 175 mm. Hg and helium of 74 mm. Hg at a total pressure of 258 mm. Hg.

All renal parameters measured during the control and experimental periods failed to reveal any deviation from accepted normals. Studies performed included renal hemodynamics utilizing inulin, PAH, and endogenous creatinine clearances; concentrating and diluting tests; and 24-hour urinary excretion of proteins. In addition, multiple determinations of blood pH and standard bicarbonate utilizing a modified Astrup technique were normal.

It was concluded that the experimental atmosphere had no adverse effect on renal function.

VARIOUS ASPECTS of renal physiology have been studied in hypobaric environments both with and without hypoxia. Lozano¹⁵ has demonstrated a mean decrease in renal hemodynamics in natives living in hypoxic environments. Studies by Berger,² Caldwell,⁴ and Axelrod and Pitts¹ have failed to demonstrate significant alterations in renal mechanisms during acute hypoxia.

In conjunction with testing proposed atmospheres for space flight, Morgan^{17,18} and Herlocher, et al.,¹² noted normal renal function. These experiments included prolonged periods of time at reduced atmospheric pressures and elevated oxygen tensions.

As discussed elsewhere in this journal, an oxygen-helium atmosphere has been proposed as an environment suitable for space flight. No detailed renal studies have been done on man in a helium atmosphere other than a 15-day experiment²⁵ with four men living in a 50 per cent oxygen, 50 per cent helium atmosphere at 7.5 psi. Throughout this study creatinine clearances and electrolyte excretory patterns remained stable. Two of the four crew members developed minimal proteinuria (1+ with a concentrated urine) which remitted at the end of the study. A large scale Navy study involving three teams of ten men, each living in 4 per cent oxygen, 76 per cent helium, and 20 per cent nitrogen at increased pressures, failed to reveal any abnormalities of any of the urine or serum parameters measured.³

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Previous work in a helium environment has shown no or minimal effects on renal function, leading to a null hypothesis. The approach in this study was to perform a careful analysis of renal function on a repetitive, serial basis. Studies included determinations of glomerular filtration rate, renal plasma flow, and concentrating and diluting mechanisms. Twenty-four hour urine protein excretion was assessed to study glomerular membrane permeability.

METHODS

Four healthy Air Force officers lived in a space cabin simulator for a period of 68 days.⁸ Initially, there was an 8-day control period followed by 56 days in a 30 per cent helium, 68 per cent oxygen environment at a total pressure of 5 psi. A 4-day postcontrol period preceded exodus from the capsule. Diet consisted of bite-sized, freeze-dried foods.¹¹

Renal function was assessed in the control and experimental periods by following multiple urine and blood studies daily and weekly (Table I). Urines were collected over 24-hour periods without added preservatives. Specimens were kept refrigerated. Electrolyte analyses were done on 4-day pools and creatinine clearances on 24-hour collections. Water balance was obtained by daily determinations of water of oxidation from food products, fluid intake, and urine and fecal losses. Glass distilled water was available ad libitum.

Sodium, potassium, chloride, and inorganic phosphorus were determined in a manner previously reported.⁵ Creatinine values were obtained using a modi-

TABLE I. SYNOPSIS OF BLOOD AND URINE STUDIES

Frequency: Daily	2X/7 Days	Urine Daily Blood 1X/7 Days	Pre-Control Post-Control
Water Intake	Urinalysis	Sodium	Inulin Clearance
Water Output	Creatinine Clearance	Potassium	Paraminohippurate Clearance
Urine		Chloride	Renal Con- centrating
Feces		BUN	Renal Diluting
		Creatinine	
		Calcium	
		Phosphorous	
		Osmolality	
		pH	
		Standard Bicarbonate	

At Selected Intervals:
(1) 24-Hour Protein
(2) Urinary Aldosterone

fication of the Folin-Wu technique¹⁰ and blood urea nitrogen by the method of Skeggs.²⁰ Both calcium and magnesium were determined utilizing the Model 214 Perkin-Elmer atomic absorption spectrophotometer with a technique developed in-house. Metabolic balances were carried out on 4-day pools of urine and feces. Methods of food and fecal analysis utilized in the balance studies are reported elsewhere in this journal.²⁴

Acid-base parameters measured included a weekly standard bicarbonate, calculated arterial pH, and alveolar partial pressure of carbon dioxide (P_{ACO_2}). Arterial pH was calculated using the Sigaard Andersen nomogram,¹⁹ alveolar P_{CO_2} , and venous blood tonometered against two known gas tensions. Determinations of pH were made on the Radiometer AME-1 Astrup machine.¹³ End tidal gases were collected in a Rahn sampler and carbon dioxide concentration was determined on the Beckman LB-1 carbon dioxide analyzer.

Renal function studies included creatinine, inulin, paramino-hippurate (PAH), and free water clearances (C_{H_2O}). Twenty-four hour creatinine clearances were used as an estimation of glomerular filtration and were performed twice weekly throughout the study. Inulin and PAH clearances were carried out during the pre- and postexperimental periods in the manner described by Homer Smith,²¹ with the exception that urinary catheters were not used. Inulin and PAH were analyzed by automated techniques.^{22,23} Since these studies were performed under an oral water diuresis, it was possible to obtain diluting ability and free water clearances. A modified 18-hour fluid deprivation test was utilized to assess the renal concentrating mechanism in the pre- and postexperimental periods. Urine and serum osmolalities were determined by the freezing point depression method with the Advanced Instrument, Inc., Osmometer, Model 31-LS.

A screening for gross proteinuria was done weekly. Twenty-four hour urinary protein excretion was measured utilizing Ames Company Combi-Stix[®] which had previously been equated with a biuret technique.⁶

At selected intervals through control and experimental periods, 24-hour urines were collected for aldosterone determinations. These were done commercially* by the double isotope dilution technique of Kliman and Petersen.¹⁴

RESULTS

Blood and Urine Electrolytes. Serum potassium, chloride, standard bicarbonate, calcium and phosphorous all showed normal values with no variation between control and experimental periods. Similar results were seen with the urinary calciums and potassium. It should be noted that subject 66 had elevated urinary calcium throughout the study and was felt to have idiopathic hypercalciuria.

Table II lists minimal but statistically significant ($p > .01$) changes in the urine sodium, chloride, phosphorous and serum sodium, and BUN. It should be noted that none of the values are outside our normal ranges and there is little change from the first control period through the experimental period.

Acid-base Studies. Standard bicarbonates, pH's, and PA_{CO_2} 's are listed in Table III. The experimental atmosphere did not significantly affect any of the parameters measured, all values being within normal limits.

Renal Function Studies. Renal clearance data and concentrating and diluting studies are summarized in Table IV. Values for inulin (glomerular filtration rate) and PAH (renal plasma flow) clearances are within accepted limits of normal,²¹ both before and

*Bio-Science Laboratories, 7600 Tyrone Ave., Van Nuys, California.

TABLE II. MEANS AND STANDARD DEVIATIONS OF BLOOD AND URINE STUDIES SHOWING SIGNIFICANT TRENDS

Subject Number	Urine Na mEq/24 hr.			Urine Chloride mEq/24 hr.			Urine Phosphorous mg/24 hr.		
	Pre-exp.	Experimental	Postexp.	Pre-exp.	Experimental	Postexp.	Pre-exp.	Experimental	Postexp.
66	120(±15)	107(±10)	88(—)	103(±26)	104(±7)	93(—)	1203(±22)	1012(±114)	967(—)
67	111(±4)	118(±14)	85(—)	96(±3)	112(±12)	81(—)	1214(±84)	1104(±59)	976(—)
68	146(±15)	117(±14)	73(—)	133(±20)	115(±14)	78(—)	1342(±112)	1071(±74)	1041(—)
69	113(±15)	96(±12)	56(—)	99(±22)	95(±12)	55(—)	1194(±9)	916(±133)	837(—)

Subject Number	Serum Na mEq/l			BUN mg%		
	Pre-exp.	Experimental	Postexp.	Pre-exp.	Experimental	Postexp.
66	145(—)	135(±3.1)	134(±5.3)	16(±1.4)	13(±0.9)	14(±0.4)
67	143(±4.2)	137(±3.5)	133(±0.4)	18(±1.8)	15(±1.4)	16(±1.8)
68	142(±5.0)	138(±3.5)	135(—)	16(±0.4)	13(±1.2)	12(±0.4)
69	142(—)	140(±3.9)	139(±5.3)	17(±1.8)	13(±1.0)	12(±1.4)

TABLE III. ACID-BASE PARAMETERS

Subject Number	Calculated Arterial pH			P_{ACO_2} mm. Hg			Standard Bicarbonate mEq/l		
	Pre-exp.	Experimental	Postexp.	Pre-exp.	Experimental	Postexp.	Pre-exp.	Experimental	Postexp.
66	7.41	7.42(±0.018)	7.38	36.8	36.5(±2.3)	38.8	23.5	23.6(±2.5)	22.5
67	7.38	7.39(±0.019)	7.39	38.3	38.9(±1.9)	38.4	23.8	23.3(±1.0)	22.3
68	7.43	7.40(±0.021)	7.37	38.2	37.5(±2.9)	39.6	24.8	23.1(±1.0)	21.8
69	7.38	7.39(±0.019)	7.39	37.0	36.8(±2.2)	36.2	22.4	22.0(±0.6)	21.5

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TABLE IV. RENAL CLEARANCES, DILUTING AND CONCENTRATING ABILITY

Subject Number		Clearances			V cc/min	Diluting		Concentrating U Osm mOsm/kg H ₂ O
		Inulin cc min/1.73 m ²	PAH	Creatinine Clearance cc/min		U Osm mOsm/kg H ₂ O	C _{H₂O} cc/min	
66	Pre	126	508	129	15.2	53	12.4	1050
	Exp			115(±15.5)				
	Post	119	478	116	13.8	62	10.8	1172
67	Pre	126	432	115	18.2	72	13.8	880
	Exp			115(±14.0)				
	Post	104	558	95	11.4	77	8.4	1078
68	Pre	99	416	125	14.4	54	11.8	1054
	Exp			121(±12.6)				
	Post	108	729	117	17.1	55	13.8	1200
69	Pre	92	576	128	16.9	47	14.1	1076
	Exp			114(±14.0)				
	Post	99	487	124	15.1	43	12.8	1042

after prolonged exposure to the oxygen-helium atmosphere. Twice weekly creatinine clearance did not show statistically significant variations between periods. Analysis of urine flow, urine osmolality and free water clearance also failed to reveal any changes. A rather wide variation in the C_{H2O} of subject 67 was thought to be related to difficulty in voiding small volumes of urine.

Following eighteen hours of fluid deprivation without exogenous vasopressin, all subjects achieved urine osmolalities of approximately 1000 mOsm/kg H₂O or greater. This degree of urinary concentration, though not maximal, reflected a normal renal concentrating mechanism.

Urine Protein Excretion. Normal 24-hour urine proteins were recorded in all the subjects throughout the study. The protein excretion ranged from 32-120 mg/24 hours.

Urinary Aldosterone and Water Balance. Twenty-four hour excretory patterns for aldosterone were all normal and correlated with urinary electrolyte studies (Table V). The steady state achieved during this study was attested to by the stable aldosterone excretory patterns.

Water-balance data are included in Table VI. It is apparent that there were definite increases in the water available for sensible and insensible loss in each subject when going to the experimental atmosphere. The mean increase in water balance was 277 cc/24 hrs with a range of 181-328 cc/day.

Metabolic Balance Studies. Metabolic balance studies were normal during this 68-day study. Table VII shows the overall normal balances of calcium, phosphorous and nitrogen. Analysis of electrolyte studies including sodium, potassium, chloride, and magnesium failed to reveal abnormalities.

DISCUSSION

It should be reiterated that in a study based on a null hypothesis, one does not expect to see clinically obvious discrepancies. For this reason it is quite important to examine the material carefully for any trends which might lead to significant problems at a later time.

Significant decreases seen in urine sodium, chloride,

and phosphorous excretion were noted mainly in the postcontrol periods, i.e., little to no change during the altitude phase of the experiment. This post-experimental decrease in electrolyte excretion was directly related to reduced dietary intake.

Reduced blood urea nitrogens (BUN) were also seen during the post-experimental period. It is felt that this was also due to the reduced dietary intake as studies have shown that variation in protein intake directly affects the BUN.⁷

Serum sodium determinations throughout the study were normal for our laboratory (131-142 mEq/l). A review of Table II shows small decreases in serum sodium from a high normal in the pre-experimental period to a low normal in the postexperimental period. The reason for this was not clear. The sodium was not surreptitiously lowered by increased levels of glucose, proteins, or lipids as all these values were normal.

TABLE V. ALDOSTERONE DETERMINATIONS
(*µg/24 hours)

Subject		Control		Experimental				
Number	Day	Number	-2	-1	+16	+30	+45	+55
66			5	14	8	12	10	21
67			24	38	14	16	14	15
68			8	17	27	9	10	16
69			9	14	9	11	11	7

*Normal = 3-32 µg/24 hours

TABLE VI. WATER BALANCE STUDIES

(Each column represents mean positive water balance in cc/24 hours)			
Subject Number	Pre-experimental	Experimental	Postexperimental
66	+1050	+1378	+1518
67	+1109	+1290	+1088
68	+1293	+1584	+1418
69	+787	+1198	+1335

TABLE VII. METABOLIC BALANCES
Intake Minus Output (gms/68 days)

Subject Number	Calcium	Phosphorous	Nitrogen
66	+0.98	+5.40	+226.4
67	-3.26	-0.27	+242.7
68	-4.34	+9.24	+294.4
69	+5.86	+6.51	+229.9

STUDY OF MAN IN OXYGEN-HELIUM ATMOSPHERE—III. RENAL RESPONSE—GLATTE & GIANNETTA

TABLE VIII. MEAN SERUM OSMOLALITIES FOR EACH PERIOD
mOsm/kg H₂O

Subject Number	Pre-Control	Experimental	Post-Control
66	289	289	290
67	288	290	290
68	291	293	296
69	289	291	292

Table VIII shows mean serum osmolality determinations during the study, all of which are in a high normal range as opposed to a more dilute serum which might be expected if a hyponatremic state was developing. With normal renal hemodynamics and diluting studies, alterations in these mechanisms could be ruled out. Normal serum and urine osmolalities and volumes are against an "inappropriate" antidiuretic hormone mechanism. From the foregoing, it was concluded that the serum sodium changes were a normal physiologic variation.

All acid-base studies and renal function parameters were unaffected by the experimental atmosphere. Renal concentrating and diluting studies were normal in the postexperimental period, as were aldosterones.

The water-balance data were of interest and not unexpected. Accepting positive water balance as a reflection of sensible and insensible water loss, it is apparent that there was a significant increase in water loss while at altitude in the experimental atmosphere. This phenomenon has been noted in earlier studies^{9,16} and is most likely secondary to the reduced atmospheric pressures and humidity.

Table VI also shows a continuous increase in water available for sensible and insensible loss in the postexperimental period. During this time, the men were subjected to multiple maximum exercise tolerance tests with attendant increased sensible and insensible water losses.

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Study of Man During a 56-day Exposure to an Oxygen-Helium Atmosphere at 258 mm. Hg Total Pressure

IV. Selected Blood Enzyme Response

M. J. BARTEK, PH.D., FRODE ULVEDAL, PH.D., and H. E. BROWN, PH.D.

An oxygen-helium atmosphere at a total pressure of 258 mm. Hg was evaluated for 56 days with 4 normal subjects to determine what effects it had on a selected group of serum enzymes and on enzyme-mediated erythropoietic mechanisms. Weekly serum lactic dehydrogenase, lactic dehydrogenase isozymes, glutamic dehydrogenase and lipase determinations were made as well as hematocrit, erythrocyte glucose-6-phosphate dehydrogenase, glutathione and glutathione stability measurements. A 14 per cent decrease in lactic dehydrogenase was observed, as well as a slight decrease in the "heart" isozyme during the experimental period. Glutamic dehydrogenase remained well within the normal range and lipase was not detected. There was a 3.4 per cent decrease in hematocrit during the post-experimental period, with an accompanying slight increase in glucose-6-phosphate dehydrogenase, glutathione and glutathione stability. Considering that all values obtained were well within the normal range, man appears to tolerate this atmosphere quite well.

AN ATMOSPHERE which differs from the one we now breathe may affect a person in a multiplicity of ways. Hence, as much pertinent scientific information must be obtained as considered feasible from a study as comprehensive as the one outlined in the first article of this series. Determining the effect of an abnormal atmosphere on erythropoiesis and on selected serum enzymes as well as their isozymes is an area not generally included even in a clinical evaluation as detailed as this. By definition, "isozymes" are enzymes which catalyze the same chemical reaction but have dissimilar electrophoretic and chromatographic properties.

Serum enzymes have proven to be useful tools for the investigator in determining the location and severity of many different diseases. Wroblewski and LaDue¹² were among the first to show that consistently elevated activities of lactic dehydrogenase (LDH) followed myocardial infarction. More recently, the isozymes of LDH have received much attention because of their tissue specificity and hence, their value in clinical diagnosis.^{6,11} Glutamic dehydrogenase is present in normal human serum only in very low levels.⁸ This enzyme has been known to increase in serum to rela-

tively high levels in pathological conditions of the liver such as obstructive jaundice.⁸ Lipase is not normally found in serum of healthy human subjects; however, under certain pathological conditions where pancreatic damage occurs, there is an elevation of lipase in the serum.⁹ The importance of serum enzyme determinations in clinical diagnosis cannot be overestimated. In spite of the vast amount of information available relating enzyme activities to different pathological conditions, little is known of the effects of a noble gas such as helium on these enzymes.

Much of the energy required by the red blood cell is provided by the pentose phosphate pathway and any alteration in the activity of glucose-6-phosphate dehydrogenase (G-6-PD) would result in a corresponding change in the amount of energy available to the cell and possibly, in its cellular integrity.¹ The quality of reduced glutathione (GSH) would also be affected, since its presence is dependent upon reduced nicotinamide adenine dinucleotide phosphate (NADPH) and hence, on G-6-PD, a NADPH-generating enzyme.¹⁴ GSH may be responsible for maintaining sulfhydryl-dependent enzymes and other cellular constituents in their reduced states.¹⁴ Thus, determination of these substances in the erythrocytes during exposure to various atmospheres would provide valuable information concerning the overall condition of the red blood cell.

In previous experiments glucose-6-phosphate dehydrogenase levels were followed and two different trends were demonstrated. When the barometric pressure was close to ground level and the atmosphere contained nitrogen, even in the presence of increased O₂ concentration, a slight downward trend was observed.¹⁴ On the other hand, there was an upward trend in G-6-PD when the barometric pressure was low in an atmosphere essentially void of nitrogen (P_B = 258 mm. Hg, P_{O₂} = 254 mm. Hg),¹³ or with helium as the inert gas (P_B = 380 mm. Hg, P_{O₂} = 167 mm. Hg).¹⁰

Hence, the purpose of this investigation was two-fold in nature: a. To determine whether a 56-day exposure to the test atmosphere (P_{O₂} = 176 mm. Hg, P_{He} = 83 mm. Hg, P_B = 258 mm. Hg) would affect the various organs of the body to such an extent as to give rise to observable changes in the serum enzymes and LDH isozyme patterns; and b, to ascertain whether such an atmosphere would have a deleterious effect on circulating red blood cells and on glutathione, the principal, readily-available source of sulfhydryl groups (-SH) in the red cell.

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The research reported in this paper was conducted by personnel at the USAF School of Aerospace Medicine, Aerospace Medical Division, AFSC, United States Air Force, Brooks Air Force Base, Texas. Further reproduction is authorized to satisfy the needs of the U.S. Government.

METHODS

Blood, drawn by the subjects themselves, was obtained by venepuncture and placed in crushed ice until centrifugation. The total time from the venepuncture to serum recovery was approximately one hour. The serum enzymes and LDH isozymes were determined the same day on blood sera which were refrigerated until analyzed. Total LDH activity was measured spectrophotometrically according to the method of Wroblewski and LaDue¹² as modified by Bowers.³ A Beckman DU spectrophotometer with a cuvette chamber controlled at 37.5° C. was employed. The LDH isozymes were separated by disc electrophoresis on acrylamide gels according to Davis.⁴ Following electrophoresis, the gels were incubated at 37° C. for two hours in the reaction mixture described by Goldberg and Cather⁵ but modified to contain in final concentration: 0.1 M sodium L(+) lactate, 0.67 mg/ml nicotinamide adenine dinucleotide (NAD⁺), 0.46 mg/ml nitro blue tetrazolium, 0.02 mg/ml phenazine methosulfate and 0.05 M Tris buffer, pH 8.3. Serum lipase was determined according to the procedure of Seligman and Nachlas⁹ and glutamic dehydrogenase was determined by the spectrophotometric method as described by Schmidt.⁸

Red cell glucose-6-phosphate dehydrogenase (G-6-PD) activity, glutathione (GSH) and glutathione stability (GSH stab.) levels were measured on heparinized blood which was kept in an icebath until analyzed. Five milliliters of blood was obtained by venepuncture (without tourniquet) and added to tubes containing 20 units of sodium heparin per 0.1 ml solution. Time from venepuncture to analyses rarely exceeded two hours. In addition to 4 samples each during pre- and post-experiment, one blood sample per week during the experiment was analyzed. G-6-PD assays were performed according to the method of Zinkham, et al.,¹⁴ using a Beckman DU spectrophotometer. During the enzyme reaction, the temperature in the cuvettes was kept constant at 30° C., using a water jacket in the spectrophotometer. Reduced glutathione was measured on 0.4 ml of blood by the method of Beutler et al.,² and glutathione stability was measured by the same method after 0.4 ml of blood was incubated for two hours with one milligram of acetyl phenylhydrazine.

The G-6-PD results are expressed as enzyme units per 100 ml washed RBC, where one enzyme unit is defined as that amount which produces an optical density (OD) change at 340 mμ of 2.07 OD units per minute per 3.0 ml of reaction mixture at 26° C. The GSH levels are reported in milligrams per 100 ml washed RBC. Hematocrits were determined in duplicate by the microhematocrit technique and reported in per cent of packed cells to total volume.

RESULTS

The average value for total LDH decreased from 617 units/ml for the pre-experimental period to 554 for the experimental period, a drop of 10.2 per cent. There was a further slight decrease in activity during

the postexperimental period to 529. The overall change from preexperiment to postexperiment was 14.3 per cent. Because there was a considerable amount of variation in the weekly values for a given subject, this decrease in total LDH activity was not statistically significant. Total LDH levels remained well within the normal range during the entire experiment.

From Figure 1, it can be seen that the isozymes re-

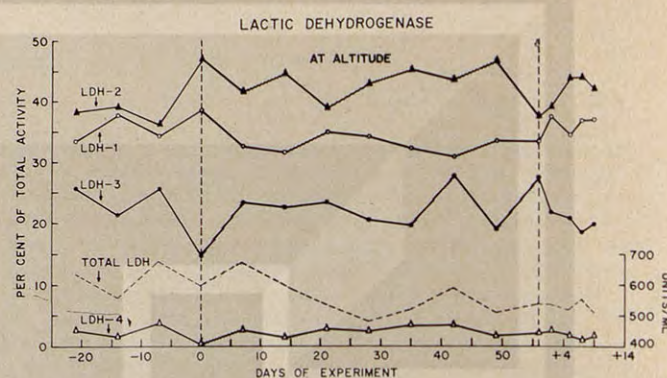


Fig. 1. Weekly means of total LDH and LDH isozymes during the pre-experimental, altitude and postexperimental phases of this study.

mained essentially constant throughout the entire duration of the experiment, with the possible exception of LDH-1, the "heart" isozyme. Isozymes were calculated on the basis of per cent of total activity. This isozyme, which had an average value of 36.0 per cent for the pre-experimental period, showed a slight but significant decrease in activity to 32.8 per cent for the experimental period. The average value for this isozyme during the postexperimental phase was 36.2 per cent; thus indicating a return to the pre-experimental average. LDH-5 was not detected during the entire experiment.

Serum glutamic dehydrogenase in human subjects exposed to an oxygen-helium environment did not increase above the normal maximum value of 0.06 units/ml (Figure 2). No detectable lipase was present in the serum during the three periods of the overall experiment.

The average value of glucose-6-phosphate dehydrogenase during the experimental period was lower than

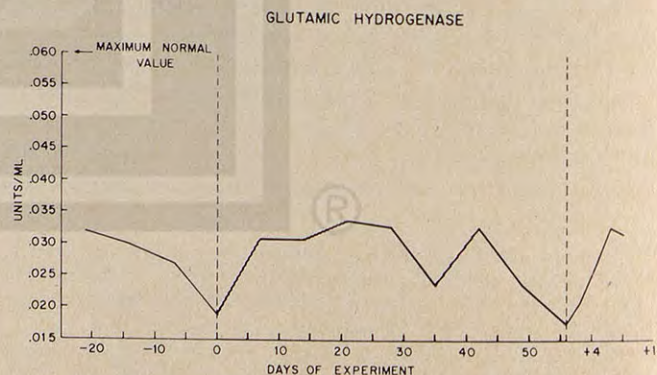


Fig. 2. Weekly means of glutamic dehydrogenase during the pre-experimental, altitude and postexperimental phases of this study.

the pre-experimental value and significantly lower than the postexperimental value (Table III). It is of interest to note that during the postexperimental period, the hematocrit dropped significantly (Table IV). Reduced glutathione levels and glutathione stability were normal both for the pre-experimental and experimental periods, but with a significant increase during the post-experimental period (Table IV).

DISCUSSION

The normal mean for total LDH, obtained from twenty-three healthy males, was 546 ± 94 enzyme units. This figure compares quite well with the value of 570 ± 58 reported by Bowers.³ One reason for the somewhat elevated values obtained during the pre-experimental period is the fact that a number of these early serum samples were slightly hemolyzed. Hemolysis may have been caused by a lack of experience on the part of the test subjects in obtaining blood by venepuncture. It has also been noted that red blood cells contain 100 times the LDH activity of normal serum.³ As the experiment progressed, the men became more proficient in this technique and hence, the amount of hemolysis in the samples decreased with a concomitant drop in total LDH activity. This decrease in total LDH with a subsequent leveling off might also indicate that the body has adapted to the new environment and the new routine of exercise, sleep and eating. The isozyme values are in close agreement with those of McVean and Rendon⁷ who used a similar technique of electrophoretic separation and enzyme assay. They too were unable to detect the presence of LDH-5. Concerning the slight decrease in LDH-1 during the altitude phase, it is interesting to speculate about the prospect of this slight decrease being related to either physical conditioning or a more constant level of exercise, producing a more steady turnover rate of cardiac muscle. Over all, this mixture of oxygen and helium at 258 mm. Hg does not cause an appreciable change in the serum levels of the enzymes studied.

Glucose-6-phosphate dehydrogenase levels were unaltered by exposure to the oxygen-helium atmosphere at 258 mm. Hg total pressure. This is in contrast to previous data¹³ obtained at the same barometric pressure but in an atmosphere of 100 per cent oxygen. Thus, it would seem that total pressure *per se* has no effect on G-6-PD. Likewise, helium shows no impact on G-6-PD levels in the red blood cell. Postexperimental values are significantly elevated, probably due to an increased rate of blood withdrawal (42 ml/day/person) during this period, in contrast with 11 and 6 ml/day/person during the pre-experimental and altitude phases, respectively. Glutathione values followed a similar pattern. No evidence for increased instability of GSH as a result of the exposure to the oxygen-helium atmosphere was apparent.

It would appear, therefore, that the red blood cell can obtain the necessary energy for maintaining cellular integrity when the individual is exposed to an atmosphere containing helium as well as in one containing nitrogen. No oxidative hemolysis was noted or expected, since the P_{AO_2} was normal.

TABLE I. SERUM LACTIC DEHYDROGENASE
(Enzyme Units* Per ml. Serum)

Period	#66	#67	#68	#69	Mean
Pre-experiment	528±139	673±76	626±67	641±119	617±62
Experiment	489±77	601±100	551±89	576±48	554±48
Postexperiment	459±21	574±36	552±61	531±18	529±50

*1 unit of LDH = Δ o.d. of 0.001/min./ml. of serum at 37.5°C.

TABLE II. SERUM LACTIC DEHYDROGENASE ISOZYMES
(Per Cent of Total Activity)

Period		LDH-1	LDH-2	LDH-3	LDH-4
Pre-experiment	$\bar{X}$	36.0*	40.1	21.9	1.9
	S.D.	0.7	1.8	3.5	1.1
Experiment	$\bar{X}$	32.8*	41.8	22.9	2.6
	S.D.	2.5	1.8	2.0	1.0
Post-experiment	$\bar{X}$	36.2	42.0	20.1	1.6
	S.D.	3.8	0.4	3.9	0.2

*P < 0.05

TABLE III. GLUCOSE-6-PHOSPHATE DEHYDROGENASE
(Enzyme Units Per 100 Ml. Washed RBC)

Period	Subject #66	Subject #67	Subject #68	Subject #69
Pre-experiment	220.35 ± 8.89	214.86 ± 31.15	218.95 ± 7.56	214.16 ± 11.50
Experiment	221.71 ± 11.68**	199.93 ± 7.38	214.37 ± 16.89**	211.72 ± 20.35
Postexperiment	239.44 ± 28.06**	200.52 ± 6.16	242.05 ± 22.46**	224.94 ± 20.39
	Pre-experiment	Experiment	Postexperiment	
	217.05 ± 16.00	211.94 ± 16.27*	226.73 ± 25.27*	

*P < 0.01

**P < 0.05

TABLE IV. GROUP MEAN VALUES FOR GLUTATHIONE,
GLUTATHIONE STABILITY AND HEMATOCRIT

Period	GSH mg/100 ml RBC	GSH Stability mg/100 ml RBC	Hematocrit
Pre-experiment	88.04 ± 13.78	84.78 ± 14.68	45.40 ± 2.72
Experiment	88.32 ± 12.67*	84.68 ± 12.89**	45.45 ± 1.22*
Postexperiment	99.58 ± 14.05*	94.38 ± 14.06**	43.89 ± 1.97*

*P > 0.01

**P < 0.01

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Study of Man During a 56-day Exposure to an Oxygen-Helium Atmosphere at 258 mm. Hg Total Pressure

V. Exercise Performance and Cardiovascular Response

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Four healthy Air Force crew members participated in an experiment to evaluate an oxygen-helium environment at 258 mm. Hg for future manned space flights. For three weeks prior to entering the space cabin simulator, the subjects took part in a program of physical exercise on a Collins bicycle ergometer. During the 56 days of the atmosphere test, they exercised 6 days per week on the same bicycle ergometer with 3 daily 20-minute exercise periods. Each exercise period was graded with a warm-up, moderate load (pulse, 150-160/min), and heavy load (pulse, 170-180/min). The workloads were determined prior to the subjects entering the chamber and remained constant. Pulse rates during moderate and heavy exercise revealed an initial fall, corresponding to conditioning and increased efficiency with the ergometer. Subsequently, these parameters leveled off except for one subject who showed a minimal rise in pulse rates during the latter half of the confinement period. Stress testing was performed immediately before and after the atmosphere test phase. Treadmill times (Balke test) showed slight improvement in the postexperimental period. Maximum oxygen consumption measurements on the bicycle ergometer postexperimentally were diminished by 2.9 to 9.4 ml/min/kg from initial values. Changes in plasma volume (pre- to post-test) ranged from -143 cc to +222 cc. Tilt table studies showed little change from earlier tests with no syncopal symptoms. It was felt that the programmed daily exercise was adequate in preventing a significant deconditioning effect or orthostatic intolerance.

PREVIOUS INVESTIGATORS have described cardiovascular deconditioning in individuals living for extended periods in space cabin simulators at dif-

ferent experimental cabin atmospheres.^{5,8,11,12,14} This deconditioning has occurred in subjects in the presence of normal gravity forces and 24-hour day cycles of sleeping, sitting, and performing minimal work. The common denominator in these studies was that of relative inactivity with isolation and confinement. Cardiovascular deconditioning was measured by the following parameters: decreased plasma volume, decreased hemoglobin and hematocrit, increased pulse rate on the Master's exercise tolerance test, decreased work capacity on the treadmill, decreased maximum oxygen consumption, and decreased orthostatic tolerance with tilt table studies.¹¹ Physiologic alterations attributed to prolonged hypodynamia and isolation in ground-based chamber studies may actually approach or equal those changes occurring with simulated weightlessness.^{2,6,12} In order to evaluate properly an experimental chamber atmosphere, the impact of deconditioning must be clearly separated from environmental effects. A graded exercise regimen to be performed within the space cabin simulator would seem to be a reasonable physiologic approach to minimize or prevent deconditioning.

During this study, four healthy volunteer subjects (ages 27-29) lived in an environment with a P_{O_2} of 175.2 mm. Hg and a P_{He} of 73.9 mm.Hg at a total pressure of 257.7 mm.Hg. Details of the total experimental profile are presented elsewhere.⁷ The individuals participated in a program of physical conditioning on a bicycle ergometer for 3 weeks prior to entering the chamber and continued on an exercise protocol throughout the entire experiment. A dual purpose was

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to determine man's ability to work in an oxygen-helium, low pressure atmosphere at graded workloads. Data from the experimental environment are compared with that obtained at ground-level conditions.

METHODS

Exercise Program. The Collins electronic bicycle ergometer* was utilized during training and throughout the atmosphere study. There were 3 daily exercise periods 5 times per week, 2 periods on the sixth day and none on the seventh. Each exercise period consisted of a graded work response as follows: 5 minutes of a warm-up workload at 75 watts, 10 minutes at a moderate workload sufficient to maintain a heart rate of 150-160 beats per minute, a 2-minute rest, and finally 5 minutes at a heavy but submaximal load sufficient to produce a heart rate of 170-180 beats per minute. The moderate and heavy workloads were determined on an individual basis in order to produce the required heart rates. The subjects pedalled at 60-65 revolutions per minute throughout. For the first two weeks of training, the workloads at the moderate and heavy levels were increased as the pulse response improved. After the initial two weeks, the workloads were held constant and remained so throughout the entire experiment.

Heart rates were monitored continuously by a cardiometer† for at least 4 exercise periods weekly. The cardiometer received its signal from a Sanborn electrocardiogram and recorded heart rate on a beat-to-beat basis from R-R interval. Miniature Beckman electrodes were positioned across the chest to obtain a maximum positive QRS deflection on the electrocardiogram and insure adequate tachometer recordings during exercise. For the purpose of data collection, heart rates were measured during the fifth minute of the warm-up, the seventh minute at the moderate workload, and the fifth minute of heavy exercise. At weekly intervals, expired air volumes were recorded with a Franz-Mueller gas meter, and 0.6 per cent of the expired gas was collected in rubber bags. A Hans-Rudolph low resistance, one-way valve was used for the expired air measurements. Sampling was performed during the sixth and seventh minutes of moderate work and the fourth and fifth minutes of heavy exercise. The collected expired gas was measured for per cent oxygen with the Beckman E-2 oxygen analyzer and for per cent carbon dioxide with the Beckman LB-1 analyzer. From the above data, it was possible to calculate minute ventilation, oxygen consumption, and metabolic rates for the moderate and heavy workloads, as described by Consolazio et al.³

Stress Testing. Work capacity was measured on the treadmill by the Balke Test.² The subject walked on a treadmill at a constant speed of 3.3 miles per hour with a one per cent (2/3 degree) increase in grade every one minute. A run was discontinued when the

subject's heart rate reached 200 beats per minute or he quit from undue fatigue. The test was performed before the start of training, before entering the experimental atmosphere, and immediately upon completion of the test phase.

Maximum oxygen consumption was measured on the Collins bicycle ergometer with the following protocol: The subjects pedalled for 5 minutes at 75 watts and for 5 minutes at their moderate workload. After a 2-minute rest, pedalling was begun at a level 30 watts greater than previous heavy workloads, with increases of 15-20 watts at 3-minute intervals. As the heart rate exceeded 190 beats per minute, the subjects increased pedal speed to 70-75 revolutions per minute and one minute expired gas collections were made. Gas sampling was by the same procedure described above. Usually, a maximum response was obtained after 5 to 7 minutes of severe exercise at heart rates between 190 and 200 beats per minute. This procedure was likewise performed before training and immediately before and after the atmospheric test phase.

Tilt table studies were performed on each of the subjects before and after the 56-day test phase. These tests for orthostatic tolerance were carried out in the Clinical Sciences Division, USAF School of Aerospace Medicine, by a method utilized for aeromedical evaluation. After a baseline was established with the individual in the horizontal position, the subject was tilted suddenly to 90 degrees. The subject, supported by a parachute harness, remained in the orthostatic position for 20 minutes. During the thirteenth and seventeenth minutes of the tilt, maneuvers of maximum breath-holding and hyperventilation-breath-holding, respectively, were performed. These maneuvers are known to produce syncope or at least stress the compensatory mechanisms of the cardiovascular system to prevent syncope. Heart rate (by electrocardiogram) and blood pressure were determined at one-minute intervals during the study. A test was terminated if presyncopal or syncopal symptoms developed.

Plasma volume studies were carried out pre- and postexperimentally utilizing I¹³¹ labeled serum albumin.¹ In addition, indirect measurements of red cell mass ("apparent RCM") were obtained by counting whole blood and subtracting plasma volume. Isotope injections were 5 uc and blood samples were obtained at 15 and 30 minutes. Subjects were in the supine position for at least 20 minutes before and throughout the blood volume studies.

RESULTS

Exercise Performance. Table I depicts the workloads performed by the four subjects throughout the entire confinement. These workloads represent an increase of 25-50 watts from those loads employed at the start of the training period. From metabolic rates determined during the moderate and heavy loads and from assuming an average of 5.5 kcal per minute for the warm-up (75 watts),¹⁵ it was possible to estimate the daily metabolic work of ergometer exercise. Subject 66 was in the best physical condition of the group and utilized

*Warren E. Collins, Inc., Boston, Mass.

†Constructed by Bioengineering, USAF SAM, Brooks AFB, Texas.

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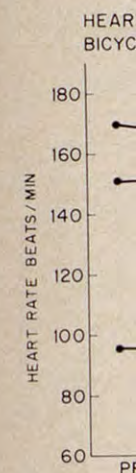


Fig. 1.
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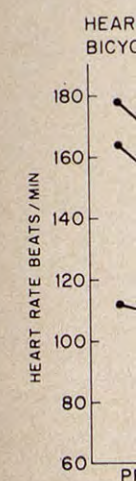


Fig. 2.
67 plotted
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TABLE I. EXERCISE WORKLOADS (WATTS) ON THE BICYCLE ERGOMETER

Subject Number	Warm-Up Workload (5 min)	Moderate Workload (10 min)	Heavy Workload (5 min)	Metabolic Work* kcal/24 hr
66	75	200	235	681
67	75	175	200	578
68	75	175	210	557
69	75	180	230	578

*Values represent metabolic work determined during the experimental phase.

approximately 681 kcal per day during the programmed exercise. Metabolic work for the other three individuals ranged from 557 to 578 kcal per day.

Subject 66 showed a slight increase in heart rate (8-13 beats per minute per metered load) during the second half of the experimental phase (Figure 1), indicating a detectable but probably insignificant amount of deconditioning. The remaining three subjects ex-

hibited a decline in heart rate per given load during the initial 1-2 weeks of the experimental phase, with fluctuation but no deconditioning trend during the remaining time of confinement (Figures 2, 3, and 4). Subjects 67, 68, and 69 also showed a slight increase (4-10 beats/min per metered load) from the eighth experimental week to the post-experimental period. Basal heart rates obtained on awakening showed only minor fluctuation without significant increases throughout the confinement period. Oxygen consumption determinations and metabolic rates for moderate and heavy exercise from the last month of the test phase were not statistically different from corresponding measurements obtained during the ground-level post-experimental period (Tables II and III). Values obtained at the low pressure altitude showed a wide fluctuation, probably related to the inherent difficulty of passing the gas-filled syringes from hypobaric to ambient pressure atmospheres for gas analysis. Sub-

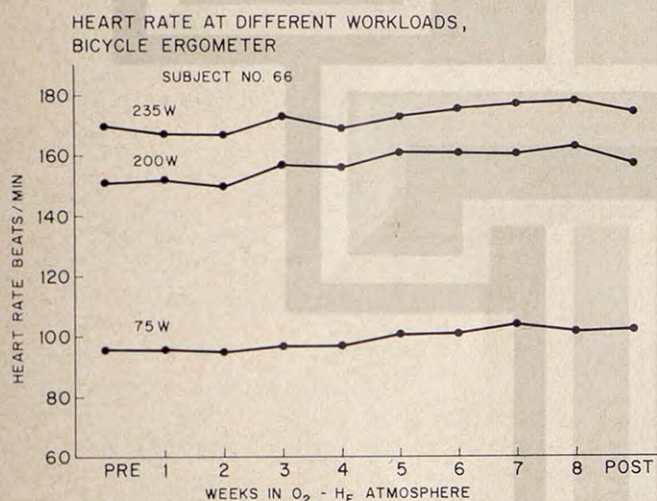


Fig. 1. Heart rate response to graded workloads for subject 66 plotted against time. Each data point represents a mean of at least 4 determinations obtained with the cardiometer.

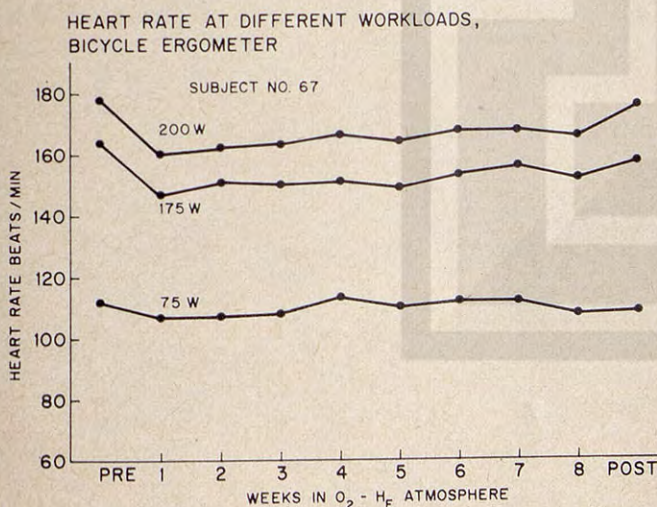


Fig. 2. Heart rate response to graded workloads for subject 67 plotted against time. Each data point represents a mean of at least 4 determinations obtained with the cardiometer.

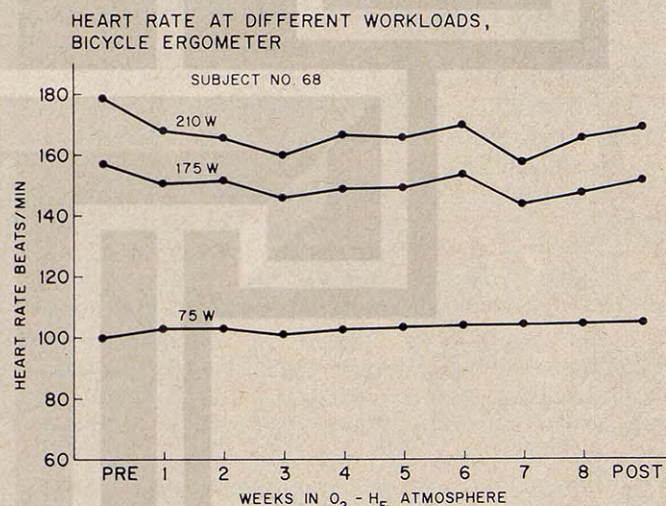


Fig. 3. Heart rate response to graded workloads for subject 68 plotted against time. Each data point represents a mean of at least 4 determinations obtained with the cardiometer.

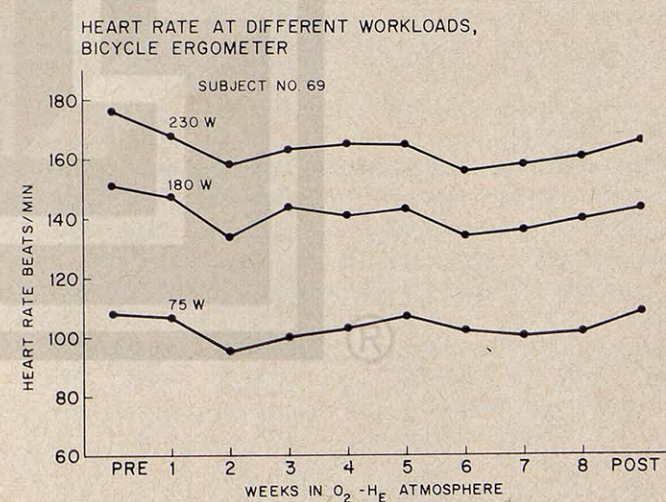


Fig. 4. Heart rate response to graded workloads for subject 69 plotted against time. Each data point represents a mean of at least 4 determinations obtained with the cardiometer.

ject 68 exhibited greater minute volumes with heavy exercise at ground level than at the experimental conditions.

Stress Testing. Table IV shows the treadmill times from the Balke test with corresponding heart rates. All four subjects showed improvement in work capacity during the 3-week training period. Three individuals actually improved their performance post-experimentally and one showed little change from pre-experimental values. Maximum oxygen consumption, measured on the bicycle ergometer, showed some decrease in all four subjects. When these values are corrected for body weight, the decrement is less and ranges from 2.9 to 9.4 ml/min/kg (Table V). The change is statistically significant in subjects 68 and 69 at the 5 and 2 per cent levels, respectively. The fall in maximum oxygen consumption is primarily a result of a decrease in minute ventilation (Table VI), rather than a decrease in oxygen extraction ability.

The results of pre- and postexperimental blood volume studies are presented in Table VII. Three subjects exhibited a slight increase and one subject a slight decrease in plasma volume, changes compatible with normal variation and the inherent error of the technique. Indirect determination of red cell mass showed no significant changes.

When baseline data are considered, tilt table studies

TABLE II. EXERCISE RESPONSE AT MODERATE WORKLOADS*

Subject Number		$\dot{V}_{O_2}$ l/min STPD	MR kcal/min	$\dot{V}_E$ l/min BTPS
66	Exp	2.63±.14	12.97±.65	67.06± 3.72
	Postexp	2.49±.00	12.54±.03	66.97± 6.47
67	Exp	2.13±.33	10.78±1.12	76.77±10.27
	Postexp	1.98±.03	10.09±.11	65.25±11.66
68	Exp	1.91±.50	10.04±2.47	59.00± 2.54
	Postexp	1.88±.04	9.68±.38	62.66± 1.35
69	Exp	2.06±.31	10.11±1.43	55.34± 5.40
	Postexp	1.98±.09	9.83±.42	52.85± 4.54

*Values represent means and S. D. of 4 determinations during the final 4 weeks of the experimental phase and of 2 determinations postexperimentally.

TABLE III. EXERCISE RESPONSE AT HEAVY WORKLOADS*

Subject Number		$\dot{V}_{O_2}$ l/min STPD	MR kcal/min	$\dot{V}_E$ l/min BTPS
66	Exp	2.90±.20	14.36±.76	80.57±4.18
	Postexp	2.96±.18	14.87±1.02	83.18±2.57
67	Exp	2.32±.26	11.88±.81	82.91±5.54
	Postexp	2.35±.05	11.99±.56	82.53±7.90
68	Exp	2.32±.43	11.76±1.71	78.60±3.70
	Postexp	2.31±.03	11.93±.05	87.97±1.92**
69	Exp	2.55±.09	13.01±.55	79.17±6.72
	Postexp	2.58±.02	13.20±.05	75.55±1.24

*Values represent means and S. D. of 3-4 determinations during the final 4 weeks of the experimental phase and of 2 determinations postexperimentally.

**p < .05

TABLE IV. TREADMILL STUDIES (BALKE TEST)

Subject Number	Pretraining		Pre-experimental*		Postexperimental*	
	Time (min)	Heart Rate (beats/min)	Time (min)	Heart Rate (beats/min)	Time (min)	Heart Rate (beats/min)
66	21	200	25	200	26	200
67	16	200	21.5	196	23	200
68	17	200	19.5	201	19	201
69	19	194	20	200	23	200

*Mean values of duplicate tests.

TABLE V. MAXIMUM OXYGEN UTILIZATION (ml/min/kg) ON THE BICYCLE ERGOMETER

Subject Number	Pretraining	Pre-exp*	Postexp*	Change	Significance
66	45.0	56.8±5.9	53.9±5.8	-2.9	NS
67	32.2	52.8±3.5	49.2±3.7	-3.6	NS
68	34.1	49.2±4.9	39.8±.9	-9.4	p<.05
69	40.4	47.7±3.0	41.3±1.3	-6.4	p=.02

*Means and S. D. of 3 evaluations.

TABLE VI. EXPIRATORY MINUTE VOLUMES (l/min BTPS) AT MAXIMUM EFFORT

Subject Number	Pretraining	Pre-experimental*	Postexperimental	Significance
66	70.49	133.75±12.33	134.20±15.50	NS
67	69.69	122.35±13.46	126.74± 2.40	NS
68	87.38	142.71±14.99	131.55±20.56	p<.1
69	87.13	125.09± 6.91	100.06± 6.32	p<.01

*Means and S. D. of 3 evaluations.

TABLE VII. I^{131} BLOOD VOLUME STUDIES (cc)

Subject Number	Pre-experimental*		Postexperimental*		Change	
	PV	RCM	PV	RCM	PV	RCM
66	3190±114	2200± 18	3412± 57	2131±74	+222	-69
67	3082±204	2050± 11	2939± 43	2072± 6	-143	+22
68	3501± 95	2336±111	3558± 1	2318±40	+ 57	-18
69	3264± 96	2220± 19	3437±145	2301±70	+173	+81

*Mean values and range of duplicate determinations.

TABLE VIII. TILT TABLE STUDIES—HEART RATE (beats/min)*

Subject Number		Baseline	12 min	16 min	20 min
66	Pre-exp	64± 6	80±13	87±11	81± 8
	Postexp	69± 6	79± 1	80± 2	84± 7
	Difference	+ 5	- 1	- 7	+ 3
67	Pre-exp	78± 3	97± 7	99± 5**	93
	Postexp	86±11	110±11	102± 1	104± 2
	Difference	+ 8	+13	+ 3	+11
68	Pre-exp	74± 3	98± 1	99± 2	99± 6
	Postexp	75± 6	97± 7	99± 9	99± 9
	Difference	+ 1	- 1	0	0
69	Pre-exp	79± 5	96± 5	101± 3	101± 4
	Postexp	69± 3	83± 6	90± 8	93± 6
	Difference	-10	-13	-11	- 8

*Mean values and range for duplicate evaluations.

**Presyncopal symptoms of pallor and nausea at heart rate of 104 beats per minute.

TABLE IX. TILT TABLE STUDIES—PULSE PRESSURE (mm. Hg)*

Subject Number		Baseline	12 min	16 min	20 min
66	Pre-exp	65±11	61±17	56±8	51±7
	Postexp	62±8	46±4	56±0	55±7
	Difference	+3	-15	0	+4
67	Pre-exp	52±8	45±11	48±18	40
	Postexp	65±11	34±4	49±1	44±4
	Difference	+13	-11	+1	+4
68	Pre-exp	34±4	29±7	27±3	20±6
	Postexp	35±7	28±4	41±5	26±8
	Difference	+1	-1	+14	+6
69	Pre-exp	55±5	55±5	31±9	38±4
	Postexp	32±12	26±2	30±10	19±5
	Difference	-23	-29	-1	-19

*Mean values and range for duplicate evaluations.

pre- and postexperimentally indicated no evidence of orthostatic intolerance. For the purpose of comparison, 12-, 16-, and 20-minute values for heart rate and pulse pressure were arbitrarily chosen (Tables VIII and IX).

Clinical Findings. Subject 69 experienced paresthesias in the lower extremities during exercise. They were described as tingling sensations and recurred throughout the entire oxygen-helium experimental period. The symptoms were minimal and occurred primarily in the legs — rarely in the trunk and upper extremities. Usually, the paresthesias were present in the first 2 to 3 minutes of the moderate workload, persisted for about 3 minutes, and disappeared during the course of moderate exercise. The same individual noted more generalized paresthesias on first ascending to the oxygen-helium atmosphere after pre-breathing 100 per cent oxygen by mask for 4 hours. Subjects 67 and 68 described similar lower extremity paresthesias with moderate exercise during the first 3 days of the experimental phase. These symptoms never occurred at ground-level conditions and were attributed to hyperventilation. Subjects 66 and 69 had rare isolated premature ventricular contractions during heavy exercise at both ground-level and experimental conditions. There were no other symptoms or signs noted with exercise during the experiment.

DISCUSSION

The exercise training program enabled the subjects to enter the space cabin simulator in good physical condition. The individuals were highly motivated and complained only of lower extremity muscle soreness during the first week of training. This was understandable in light of increasing workloads in persons not ordinarily accustomed to such strenuous leg exercise. The Collins bicycle ergometer was quite suitable both for training and for the exercise protocol during the confinement.

All four individuals stated that pedalling the ergometer was less difficult in the hypobaric oxygen-helium atmosphere than at ambient conditions. Decreased density of the experimental environment with diminished work of breathing might explain the subjective

impressions. In addition, possibly mechanical factors such as diminished friction with pedalling of the ergometer might have been operative. Three subjects showed an initial decrease in heart rate per metered load during the first 2-3 weeks of the experimental phase, which was interpreted as continued efficiency on the ergometer. The same individuals demonstrated a 5-10 beats per minute increase in heart rate per given workload from the last experimental week to the postexperimental control period, which also appears to support the subjective impressions. However, when oxygen consumption determinations and metabolic rates at moderate and heavy workloads from the experimental period are compared with data obtained at postexperimental ground-level conditions, no detectable statistical difference is noted.

The subjects were able to work in the oxygen-helium, 258 mm. Hg atmosphere without significant medical difficulties. The lower extremity paresthesias, noted initially in three individuals and recurrently in one, were most likely due to hyperventilation. These symptoms were not bothersome and did not prevent or interfere with the subjects' ability to work. There were no joint problems with exercise or neurologic symptoms which might have been suggestive of a decompression disturbance.

Lamb, et al.,^{10,11} have shown that physical inactivity, uncomplicated by alteration of the body's orientation to normal gravity forces, can lead to cardiovascular deconditioning. Strict chair rest for 4 days produced symptomatic orthostatic intolerance in five out of six normal individuals.¹⁰ Several investigators have described diminished work performance and inadequate orthostatic reflexes in persons living for various periods, confined to space cabin simulators.^{8,11,12,14} In the present study, work capacity on the treadmill Balke test actually increased slightly in three subjects and was unchanged in a fourth after 56 days of confinement in the experimental atmosphere. Although maximum oxygen consumption fell somewhat in all four individuals, the decrease was statistically significant in only two and postexperimental values approached pretraining levels in only one subject. The decrement in maximum oxygen consumption was mostly at the expense of minute ventilation and not of oxygen extraction. Tilt table studies after confinement demonstrated no evidence of orthostatic intolerance. In fact, one subject (67) who exhibited presyncopal symptoms prior to the experimental phase was asymptomatic postexperimentally.

Physiologic deconditioning results in a diminished plasma volume and red cell mass.⁹ The present studies revealed only minor postexperimental fluctuations of plasma volume within the error of the I¹³¹ isotope dilution method. Actually, by this method, three subjects showed slight increases in plasma volume. Indirect determinations of red cell mass demonstrated no change from pre-experimental values. The exercise protocol utilized appeared to be adequate in maintaining the body's homeostatic mechanisms for preventing appreciable change in blood volume compartments.

Lamb⁹ has proposed that acclimatization to hypoxia causes cardiovascular compensatory mechanisms to pre-

vail which are directly opposite to changes induced by inactivity. He suggested that prolonged hypoxia, equivalent to an altitude of 10,000 feet, would be useful in preventing deconditioning during manned space flight. It would appear that a graded exercise program would be a more normal physiologic stress than hypoxia in preventing the deterioration of cardiovascular responses. The present work has shown that a rather severe program of ergometer exercise prevented deconditioning in a normal gravity environment. Whether such a protocol would be adequate during manned space flight, when the problem of weightlessness is added to inactivity, remains uncertain. Stevens and Lamb¹³ have presented evidence that lower body negative pressure in the supine position produces changes similar to upright tilting. Recently, an instrument that combines lower body negative pressure with a pedal ergometer has been devised and is currently being evaluated.⁴

In conclusion, an exercise protocol on a bicycle ergometer was adequate in preventing cardiovascular deconditioning in four subjects living for 56 days in a hypobaric oxygen-helium environment. Although normal gravity stresses were present, cardiovascular deterioration from relative inactivity and confinement was prevented. It is suggested that a similar exercise protocol, possibly combined with lower body negative pressure, be considered for future long-term manned space flight.

ACKNOWLEDGMENT

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Study of Man During a 56-day Exposure to an Oxygen-Helium Atmosphere at 258 mm. Hg Total Pressure

VI. Excretion of Steroids and Catecholamines

FRODE ULVEDAL, PH.D., and ANN J. ROBERTS, B.A.

The urinary excretion of 17-hydroxycorticosteroids, corticosterone-like hormones, epinephrine and norepinephrine was measured in an attempt to evaluate the degree of emotional and physical stress experienced by subjects participating in an oxygen-helium atmosphere experiment at 258 mm. Hg total pressure for 56 days. The values obtained for these variables were within physiologic range for this type of experiment, but several trends were observed. Differences in the rest-day values vs. work-day values were noted. A reversal in the normal 12-hour excretion ratio took place for the subjects who slept during the days and worked during the nights, as measured by the 17-OHCS and corticosterone-like hormones. The effect of diet and exercise in this environment was also reported.

Thus, it appears that the oxygen-helium atmosphere utilized in this experiment provides satisfactory environmental conditions for human activity over a prolonged period of confinement.

A VARIETY of disturbing or provoking factors, either emotional or physical, may act as stress stimuli manifesting themselves by increased adrenal output. The emphasis placed on the importance of the central nervous-adrenomedullary system and on the pituitary-adrenocortical system varies; undoubtedly both of these systems function in determining the organism's reaction to stress.^{7,18} However, the response to some stimuli involves only the adrenomedullary system, others the adrenocortical system, and some stimuli invoke mixed reactions.²⁰ There are also considerable individual differences in the response to specific stimuli.^{7,9} Because of this divergence in response to stress, the urinary excretion of 17-hydroxycorticosteroids, corticosterone-like hormones, epinephrine and norepinephrine was used to evaluate the effects on subjects exposed in an oxygen-helium atmosphere at reduced pressure for 56 days, where in addition to the emotional factors, assessment could be made on the effect of reversal in the normal sleep-work schedule, and the effect of exercise during these conditions.

METHODS

The four-man double-walled space cabin simulator,

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The research reported in this paper was conducted by personnel of the Environmental Systems Branch, USAF School of Aerospace Medicine, Aerospace Medical Research Division, AFSC, United States Air Force, Brooks AFB, Texas. Further reproduction is authorized to satisfy the needs of the U. S. Government.

its life support system, equipment, and experimental design have been discussed elsewhere.¹

The study to be discussed here concerns four subjects who participated in an oxygen-helium atmosphere at 258 mm. Hg pressure for 56 days. The four subjects were combat crew members from 27 to 29 years of age; two of the subjects were pilots and the other two were navigators. The profile of this experiment has been previously described.¹⁰ There were control periods outside the chamber as well as inside the chamber, both before and after the altitude phase of the experiment. The subjects spent a total of 68 days in the chamber. Upon arrival, the subjects were started on an exercise schedule, where they rode a bicycle ergometer for 20 minutes, three times per day, Mondays through Fridays. The schedule called for one exercise period on Saturdays, and complete rest on Sundays. Fifteen days prior to the start of the pre-experimental period, the exercise load was stabilized for three of the subjects at 568 kcal per day and for the fourth at 681 kcal per day; this was based on the heart rate achieved by the subjects.²² This exercise regimen continued until the post-experimental period.

The diet used in this experiment consisted of bite-size dehydrated food, calculated to give each subject 45 kcal/kg lean body weight/day, plus 600 kcal/day for the exercise regimen. Solid food was supplemented with fruit juices and coffee or tea. In addition to the minerals and vitamins, the daily composition of this food was approximately 15 per cent protein, 65 per cent carbohydrates and 20 per cent fat.¹¹

The environmental and atmospheric parameters while the subjects were inside the space cabin simulator have been reported elsewhere.¹⁰ During the two control periods the atmospheric composition was essentially normal for San Antonio, Texas, while during the experimental phase the atmosphere contained 28.7 per cent helium and 68.3 per cent oxygen; the other gases were kept at a minimum. It should be noted that the 68.3 per cent oxygen provides an alveolar oxygen partial pressure approximately equal to ground level conditions. The chamber temperature was selected by the subjects while the humidity, the gaseous atmosphere and the pressure were controlled from the outside.

Before, during, and after the experiment, each subject collected urine in 12-hour pools. The first collection period started at 8:00 a.m. and lasted until 8:00 p.m. The second period was from 8:00 p.m. to 8:00 a.m. the following morning. In this manner, the di-

urnal cycle, as well as the total 24-hour output could be studied. Regardless of the sleep schedule, each 12-hour pool contained the urine from an 8-hour sleep period. Upon voiding, the urine was measured, and 10 per cent of each voiding added to glass bottles containing HCl acid. During the entire period from voiding to analysis, which was immediately after each 24-hour period, the urine was kept refrigerated (4°C.). Corticosterone-like hormones and 17-OHCS were assayed on 12-hour samples.

The steroids were extracted in butanol,³ and 17-OHCS were determined on a portion of this extract using Porter-Silber's method which was selected because it was specific for the dihydroxyacetone side chain. Corticosterone-like hormones were also analyzed on this extract by using Mader and Buck's bluetetrazolium method¹³ which was selected because it reacts with an alpha ketone, but does not require the presence of a hydroxyl group on C₁₇. By using these two methods it was felt that a more complete picture of the physiologically active steroids would be obtained.

Epinephrine and norepinephrine were assayed on 24-hour urine samples by using the trihydroxyindol method of von Euler.⁶ The accuracy and precision of the assays were the same as reported earlier from this laboratory.²⁰

The determinations of these four variables were performed on urine collected from Sunday mornings to Monday mornings, when the subjects had no exercise and a minimum of activity, and on urine collected from Wednesday mornings to Thursday mornings, a period in the middle of the week with maximum activity and exercise.

RESULTS

Summary of Individual Variables

17-OHCS. Period by period, the values for these hormones are statistically different. All the values are within normal limits as experienced in our laboratory, except for the value obtained during the postexperimental period inside the chamber (Table I). During the postexperimental period, when the subjects were back to normal living, the group mean value was normal. It is of interest that the values progressively increased from the time the subjects entered the chamber until they came out.

Corticosterone-like Hormones. The only significant

TABLE I. SUMMARY OF THE STRESS VARIABLES

Period	Corticosterone-like			
	17-OHCS (mg/24 hr)	Hormones (mg/24 hr)	Epinephrine (μg/24 hr)	Norepinephrine (μg/24 hr)
Pre-Exp Out	8.46 ± 2.38	56.68 ± 16.60	16.41 ± 7.87	68.76 ± 12.07
Pre-Exp In	6.21 ± 1.59	41.87 ± 12.32	12.51 ± 5.09	65.11 ± 21.07
Experiment	7.86 ± 1.90	47.31 ± 14.87	14.64 ± 8.34	45.80 ± 18.68
Postexp In	10.31 ± 2.84	43.66 ± 7.50	20.03 ± 6.38	54.38 ± 12.72
Postexp Out	5.74 ± 2.15	47.21 ± 26.90	17.40 ± 7.88	84.35 ± 24.62
LEVEL OF SIGNIFICANCE				
Pre Out vs Pre In	P<0.01	P>0.01	P<0.05	P<0.5
Pre In vs Exp @ Alt	P>0.01	P<0.1	P<0.2	P>0.01
Exp @ Alt vs Post In	P>0.01	P<0.1	P<0.01	P<0.01
Post In vs Post Out	P>0.01	P<0.5	P<0.3	P>0.01
Exp @ Alt vs Post Out	P>0.01	P<0.9	P<0.2	P>0.01

change in excretion of the corticosterone-like hormones occurred when the subjects went from pre-experiment outside to pre-experiment inside the chamber. The value obtained when the subjects were exposed to the experimental environment was higher than either of the control periods inside the chamber; the experimental pressure and atmosphere may therefore have a slight effect on this hormone group as seen by the data in Table I. The values for the last control period seem to return toward the initial control level.

Epinephrine. All the epinephrine values are higher than normally seen in this laboratory for similar subjects ($6.4 \mu\text{g} \pm 2.9/24 \text{ hrs}$). This is probably the result of the high exercise level maintained by the subjects.

There was a significant decrease when the subjects entered the chamber, with a slight increase during the experimental phase. However, both postexperimental periods were higher than the experimental period. This indicates that the experimental phase is not as stressful as the control medical testing period, when repetitive tilt table, work capacity, maximum oxygen consumption, etc., tests were performed.

Norepinephrine. What was said for epinephrine can also be said for norepinephrine, even though the pattern seems to be different. The control periods were significantly higher than the experimental period, but this can be attributed to the additional level of activity (like treadmill and walking) performed during these periods.

The hormone values obtained for the individual subjects are summarized, by period, in Table II. It should be pointed out that subjects #66 and #69 were teammates and slept during the period of 12:00 noon to 8:00 p.m.

Diurnal Variation of Steroid Excretions. It should be re-emphasized that 8 days prior to the altitude phase of the experiment, the subjects entered the cham-

TABLE II. SUMMARY OF THE STRESS VARIABLES FOR THE INDIVIDUAL SUBJECTS

Period	Subject #66	Subject #67	Subject #68	Subject #69
17-OHCS (mg/24 hr)				
Pre Outside	8.11 ± 2.41	7.30 ± 1.92	9.24 ± 1.34	7.85 ± 2.03
Pre Inside	4.81 ± 0.93	4.93 ± 0.55	7.33 ± 0.69	7.78 ± 1.17
Altitude	7.67 ± 1.77	7.77 ± 1.79	7.94 ± 2.05	8.38 ± 2.14
Post Inside	10.67 ± 3.46	10.14 ± 4.17	9.97 ± 2.83	10.48 ± 2.54
Post Outside	3.96 ± 1.21	4.75 ± 2.56	5.44 ± 2.16	4.22 ± 2.72
Corticosterone-like Hormones (mg/24 hr)				
Pre Outside	59.70 ± 17.30	55.10 ± 19.50	44.48 ± 7.32	67.41 ± 14.58
Pre Inside	40.42 ± 8.18	53.73 ± 8.93	33.40 ± 9.32	39.92 ± 16.65
Altitude	38.21 ± 5.66	59.83 ± 13.53	40.84 ± 9.39	50.33 ± 17.71
Post Inside	42.00 ± 7.49	51.84 ± 1.77	38.56 ± 2.16	42.25 ± 9.94
Post Outside	60.33 ± 19.92	54.36 ± 14.15	36.36 ± 8.37	45.44 ± 6.17
Epinephrine (μg/24 hr)				
Pre Outside	15.62 ± 8.10	20.85 ± 6.96	9.36 ± 6.09	19.64 ± 6.97
Pre Inside	12.89 ± 7.75	15.03 ± 6.26	10.38 ± 2.03	11.72 ± 4.69
Altitude	14.14 ± 7.04	19.01 ± 8.61	8.55 ± 5.31	16.84 ± 8.62
Post Inside	24.46 ± 7.21	22.40 ± 5.52	12.88 ± 2.44	20.38 ± 4.57
Post Outside	25.05 ± 9.54	14.90 ± 6.60	11.24 ± 3.12	18.41 ± 5.31
Norepinephrine (μg/24 hr)				
Pre Outside	70.14 ± 16.58	73.38 ± 8.36	61.95 ± 11.51	69.71 ± 11.54
Pre Inside	52.34 ± 27.11	78.22 ± 26.67	59.01 ± 5.39	70.87 ± 18.93
Altitude	33.18 ± 13.21	53.03 ± 21.87	43.76 ± 14.75	52.63 ± 14.49
Post Inside	60.64 ± 14.86	58.40 ± 8.41	45.73 ± 13.12	52.76 ± 15.06
Post Outside	103.19 ± 26.08	102.56 ± 17.58	67.38 ± 12.69	64.29 ± 6.43

ber and start new sleep cycle the entire experimental ground-level periods also main

The daily sleep (#69) to sleep other two subjects midnight to 8

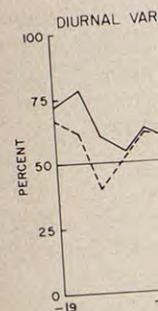


Fig. 1. The from 8 a.m. to 8 p.m. hour period.



Fig. 2. The hormones shown of the total

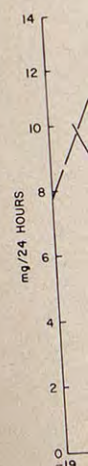


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1.54 ± 6.97
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1.38 ± 4.57
1.1 ± 5.31

1 ± 11.54
1.7 ± 18.93
1.3 ± 14.49
1.5 ± 15.06
1.9 ± 6.43

ber and started the daily schedule (including the new sleep cycle), which was to be kept throughout the entire experiment. There was a similar 4-day ground-level period postexperimentally, when the subjects also maintained the experimental daily schedule.

The daily schedule allowed two subjects (#66 and #69) to sleep from 12:00 noon to 8:00 p.m., and the other two subjects (#67 and #68) to sleep from 12:00 midnight to 8:00 a.m. The latter two therefore had a

normal sleep schedule.

Figure 1 shows the summary of the excretion of 17-OHCS reported as the excretion from 8:00 a.m. to 8:00 p.m. in per cent of the total excretion for the 24-hour period.

Normally the excretion during this 12-hour period varies from 57-70 per cent, and this holds true, throughout, for the two subjects on the normal sleep cycle. The two subjects who slept from noon to 8:00 p.m. had different patterns, however. In the process of adaptation to the new sleep cycle, these two subjects were under physiologic stress. The resulting increased output of 17-OHCS during this 12-hour period (when they should be asleep) reached a peak about the 6th day, then decreased until the 15th to 16th day, when the normal excretion pattern was reversed. From then on, with one exception, the excretion stayed below the 50 per cent line — including the postexperimental period inside the chamber. The normal diurnal pattern reverted back to normal by the second day on normal sleep cycle. An interesting observation is that, except for the initial period of adaptation, the total output in mg/24 hrs did not change greatly for these particular subjects (#66 and #69), indicating that complete adaptation had taken place.

The exceptions seen during the pre- and post-experimental periods outside were observed when the subjects were not under rigid restrictions.

Figure 2 shows the change in diurnal rhythm for the corticosterone-like hormones, which is essentially the same pattern as observed for the 17-OHCS. A few differences are apparent. The initial peak at the beginning of the new sleep cycle is absent, and the downward trend begins about one week earlier than for the 17-OHCS. The return to the normal diurnal cycle appears to be slower in the corticosterone-like hormones than for the 17-OHCS. Table III summarizes the diurnal variations by periods.

Steroid Excretion During a Workday Versus a Rest-day. The expected trend of excretion for the 17-OHCS was observed (Figure 3). It showed an increase during the initial period of medical testing, the process of learning chamber procedures and the anxiety anticipating the experiment. A peak occurs also during the first part of the experimental phase. Halfway through the experiment (31 to 41 days) a peak corresponding to a period of lowered subject morale was seen. Excretion values changed after the subjects were given additional stimulus to continue the experiment. The last peak is apparently related to the elation of coming down and the successful completion of the experiment.

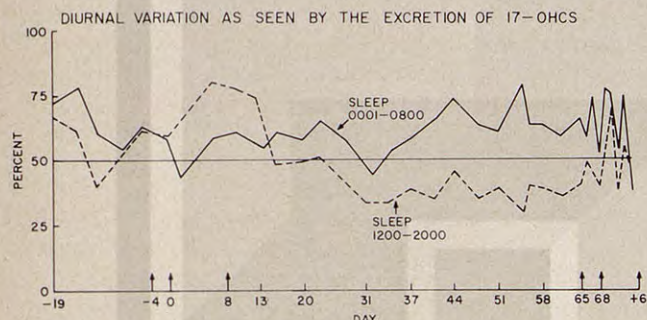


Fig. 1. The excretion of 17-OHCS reported for the excretion from 8 a.m. to 8 p.m. in per cent of total excretion for the 24-hour period.

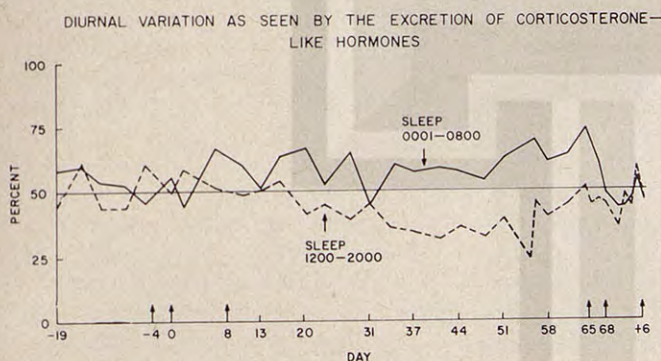


Fig. 2. The excretion pattern for the corticosterone-like hormones shown for the excretion from 8 a.m. to 8 p.m. in per cent of the total 24-hour period.

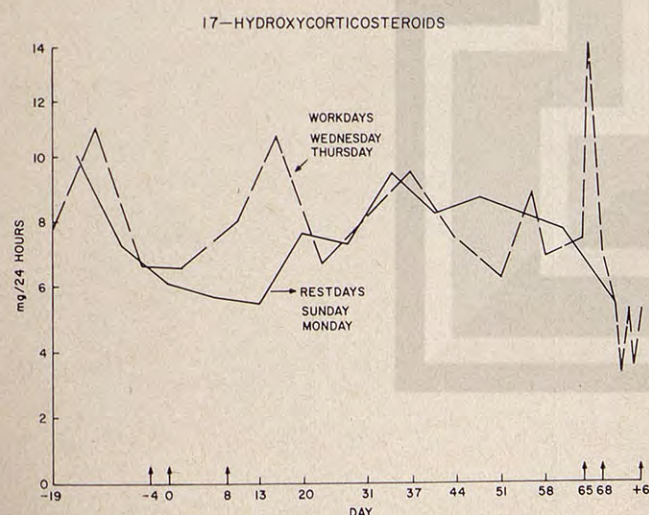


Fig. 3. Trends for the 17-OHCS excretion as seen during rest-days and workdays. Day 0 is the day when the subjects entered the chamber pre-experimentally and day 68 is the day they left the chamber post-experimentally.

TABLE III. SUMMARY OF THE DIURNAL RHYTHM FOR 17-OHCS AND CORTICOSTERONE-LIKE HORMONES

Period	17-OHCS		Corticosterone-like Hormones	
	Sleep Schedule		Sleep Schedule	
	12 noon-8 p.m.	midnight-8 a.m.	12 noon-8 p.m.	midnight-8 a.m.
Pre-exp (outside)	56.9%	65.7%	51.5%	54.2%
Pre-exp (inside)	68.2	53.4	53.2	56.1
Experiment	43.8	61.7	41.2	61.4
Postexp (inside)	44.4	62.1	45.3	58.1
Postexp (outside)	52.5	63.3	46.7	47.2

It should be noted that initially, the restday excretions are lower than weekday excretions. Several exceptions to this were seen, especially during the last half of the experiment.

Two excretion patterns for the corticosterone-like hormones were immediately evident from looking at the curves (Figure 4). While there appears to be a general downward trend in the excretion during the workdays, there is a continuous upward trend for the restday values. More significant is the fact that with only a few exceptions, the restday values are higher than the workday values. The excretion of the corticosterone-like hormones as seen during rest days was interesting, because it seems to reflect the psychologic picture better than the 17-OHCS excretion.

The abrupt decrease seen in corticosterone-like hormones after the 51st day is attributed to the announce-

ment to the subjects of an earlier than scheduled down time for the experiment.

Trends for Catecholamine Excretions. The curve for epinephrine excretion follows the typical anticipation pattern observed in the past.²⁰ The levels were high during the pre-experimental period, outside, while the subjects underwent extensive medical examinations, learning chamber procedures and general anticipation. In this experiment (Figure 5) the excretion of epinephrine was also higher than normal in our laboratory because of the added stress of exercise which undoubtedly affects the curve (normal value: 6.4 ± 2.9 $\mu\text{g}/24$ hours). The second peak occurred when the subjects entered the chamber and were taken to the experimental altitude and atmosphere. Then following a decline, there was an upward trend after one-third of the experiment was completed.

Up until this time (21st day), restday values had been lower than the workday values. For the remaining two-thirds of the experiment, the restday values were higher than the workday values. During debriefing, after the experiment, all subjects admitted that boredom on the weekends became quite severe the last two-thirds of the experiment. This was also a time when they worried about family, finances and their future. The drastic drop in epinephrine output occurred after they were informed of the early completion of the experiment. In addition, the subjects began to anticipate plans for reunion with their families.

The curves for norepinephrine were in general similar to the epinephrine curves, and again it is apparent that the restday values were higher and more stable than the workday values (Figure 6). However, a point of interest here is that the norepinephrine curves during the weekdays reflect the exercise conditioning and de-conditioning patterns observed by the heart rate and oxygen consumption data throughout the entire experiment.²⁰ Increasing heart rates during the last part of

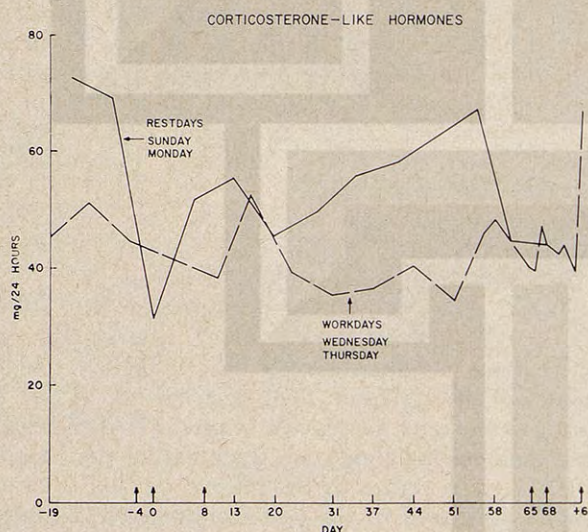


Fig. 4. Trends for the corticosterone-like hormones as observed during the entire experiment. The small arrows indicate start of diet, entry into the chamber, start of altitude phase, down from altitude, and final exit from the chamber.

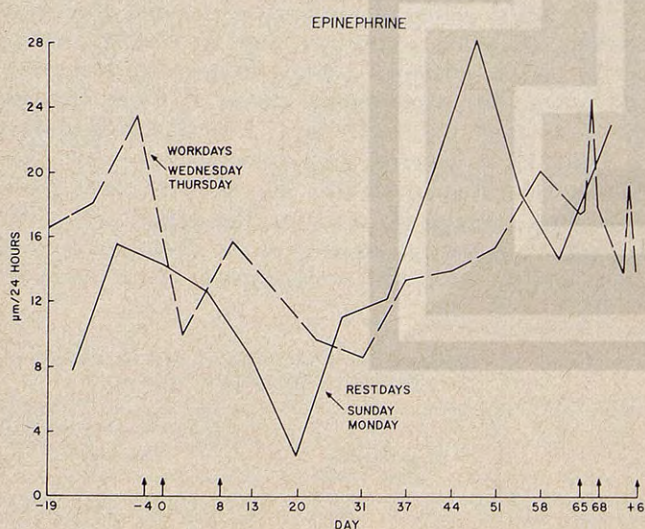


Fig. 5. Trends for the epinephrine values observed during the oxygen-helium experiment. Note the curve for restdays is different from the workdays.

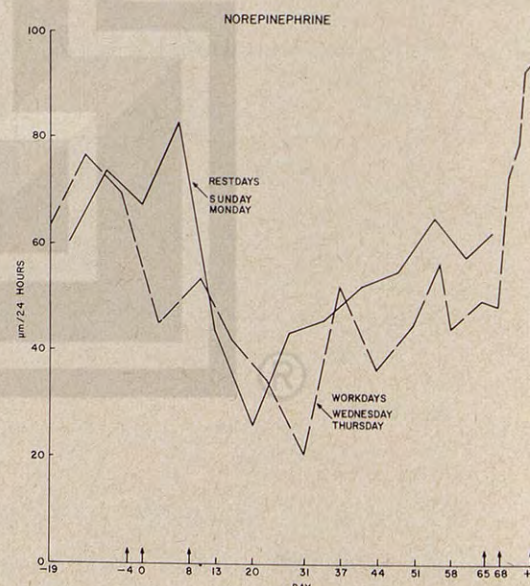


Fig. 6. Trends for the norepinephrine values observed during the experiment.

the experiment indicated that the subjects had to work harder for the same workload.

In addition to following the exercising conditioning curve, the values for norepinephrine, especially on rest-days, reflect the psychologic condition of the subjects.

DISCUSSION AND CONCLUSIONS

In earlier studies it was observed that changes in the diet produced changes in the steroid excretion.²¹ In this experiment, the change from normal diet to experimental diet coincided within four days of the subjects' entry into the chamber for the ground-level control period. Because of this, the effect of the diet change was obscured, but since the medical testing, exercise and other activities were the same in both periods, it may be that the decreases seen for these two variables might be adaptive changes caused by the diet. The diet was not very palatable and the subjects had severe reservations about the food. Likewise, the character of the food was different from the subjects' usual diet.¹¹ It was also difficult to ascertain the effect of returning to normal diet and eating habits postexperimentally. The corticosterone-like hormones showed a trend toward the pre-experiment level, while the 17-OHCS dropped to their lowest level during this period. The values obtained for the pre-diet and on-diet control periods were statistically significant, and undoubtedly, the diet did exert an influence on the excretion of these variables, both psychologically and biochemically. The diet effect seen here, even though difficult to assess, was not dissimilar to what had been reported earlier.²¹

Fifteen days prior to the start of the preexperimental period, the exercise load was stabilized for three of the subjects at 568 kcal per day and for the fourth at 681 kcal per day; this was based on the heart rate achieved by the subjects.²² As indicated earlier, this level of exercise was maintained until the postexperimental, outside control period. This was in addition to medical testing which included such stimulants as treadmill, tilt table, and EEG's. A true baseline for the steroids was not really obtained before the postexperimental period outside the chamber, when only medical testing was performed. During a similar pre-experimental period with only medical testing, we have observed excretion for 17-OHCS of 8.9 ± 2.0 mg/24 hours and 31.3 ± 8.7 mg/24 hours for the corticosterone-like hormones. As seen from the data in the summary table (Table I), the effect of additional exercises on the excretion of the steroids appeared to be absent, except for the corticosterone-like hormones which were above normal. It cannot be ascertained if this was a direct effect of the exercise regimen, or if it was caused by physiologic salt and water changes due to increased perspiration as the exercising took place. However, it should be noted that as the level of exercise proficiency was reached, there were also decreases in steroid excretions. This can also be noted from the figures displaying the trends.

There were many emotional factors which could have influenced the excretion of the stress variables.

It was therefore difficult to isolate the effect of a single factor, such as exercise. Nazar¹⁵ reported a 36.3 per cent increase in the excretion of 17-OHCS after an exercise load similar to that of our subjects. Comparing the mean value for the subjects postexperimentally outside (with no scheduled exercise) to the pre-experimental period outside, there was a 47.4 per cent increase in 17-OHCS excretion, but it should be noted that during this period the subjects were undergoing vigorous physical conditioning and training. During the altitude phase there was a 36.9 per cent increase in 17-OHCS excretion over the post-experimental period outside, which was in line with Nazar's results.

The catecholamine data reported in this study were higher than reported earlier, when no additional exercise was involved (6.4 ± 2.9 and 55.7 ± 9.9 μ g/24 hrs for epinephrine and norepinephrine, respectively).²⁰ During the initial control periods the norepinephrine values reflect the exercise conditioning of the subjects, while during the latter part this hormone reflects the de-conditioning of the subjects. This was also seen by the increased heart rate and oxygen consumption curves, which showed that the subjects had to work harder for the same workload. The norepinephrine excretion was lowest during the experimental period and it was interesting that the subjects reported that it was easier to perform the work in the environmental atmosphere at decreased pressure. The epinephrine excretion levels were different from those of norepinephrine, but also were undoubtedly influenced by the exercise regimen. Karki¹² and Rodahl¹⁶ have reported increased excretion levels of catecholamines during exercise, and again the increases observed here were of the same order of magnitude for the same workload. But again it was difficult to separate the physical stress from the emotional stress, other than to state that physical work is known to affect these variables.

Normal sleep patterns have been associated with many measurable physiologic and biochemical changes. As the body prepares for wakening, there is rapid reversal of these changes. Diurnal variations have been observed for the secretions from the adrenal glands.^{5,12,14} It is generally agreed that the urinary excretion of 17-OHCS starts to increase early in the morning, reaching a peak around 8 to 9 a.m., followed by a gradual decline until about 3 a.m. when the cycle is repeated. It has been observed that on a 12-hour basis, total excretion of this hormone group is higher during the daylight hours than at night.^{8,20}

Normal diurnal rhythms were observed in this experiment during the pre-experimental period outside the chamber; however, after approximately 10 to 16 days on the new sleep cycle, subjects #66 and #69 showed a reversal of the normal diurnal rhythm in both types of steroid analyses, based on the 12-hour excretion pattern. This appeared to be contrary to the observations made by Migeon, et al.¹⁴ This group of investigators agree with the excretion patterns observed for normal sleeping subjects. They noticed the same excretion pattern in nurses and watchmen after

they had worked the nightshift for 6 months. These subjects slept from 2 p.m. to 10 p.m., while our subjects slept from 12 noon to 8 p.m. It should be emphasized that this study did not actually delineate the curve by studying 6-8 points during the 24-hour period. By studying closely the curves from the tables in Migeon's paper, it was evident that the part of the curve when the night watchmen and nurses slept went to a much lower level than the normal curve during this period. Therefore, one can calculate that the total amount excreted during the 12-hour daylight period was lower than normal, and the per cent change for the 12-hour period also went below the 50 per cent mark. During previous experiments (17-day duration) when subjects slept 5 hours per night on a rotating schedule (one night from 9 p.m. to 2 p.m. and the next night from 2 a.m. to 7 a.m.), there were no inversions of the normal ratio, but the band continuously narrowed. During the first few days the excretion ratios were 62 per cent a.m. to 38 per cent p.m., and at the end this ratio was 56 per cent to 44 per cent.²⁰

One point in the reversal of the diurnal excretion seen by the curves for 17-OHCS and corticosterone-like hormones cannot be readily explained: Why did the corticosterone-like hormones reverse the normal pattern sooner than the 17-OHCS, and return at a slower rate? Porter Silber's reaction is essentially for steroids with the dihydroxyacetone side chain, while the steroids assayed by the bluetetrazolium technique include the 17-OHCS's possessing an α -ketone on carbon 17. One answer was that the additional steroids included by the latter technique outweighed the 17-OHCS and had different secretion patterns.

In earlier experiments of 17 days duration,²⁰ there were indications of continuous upward trends for several of the same variables reported here. In this experiment there were some trends which can be explained, and there were some trends which cannot be readily explained. It appeared that boredom definitely played a role as seen by the restday values, which showed a distinct curve in three of the four variables (Figures 4, 5, 6). The subjects should always be well-informed, as evidenced by the fact that as soon as the subjects were told about the termination date, there was a definite improvement in morale, and also a drop in these variables.

Several investigators have reported on steroid excretion as related to emotional stress. Pre-operative anxiety of hospital patients caused a rise in their steroid levels. A corresponding decrease was seen in the steroid level of postoperative patients. This was interpreted as the removal of the psychologic stress by the anesthesia.⁴ Additional evidence of the importance of psychologic stress in steroid excretion was shown by experiments that involved viewing films of various emotional impacts. Emotion-laden films caused elevation in the 17-OHCS levels, while films of a bland nature caused decreases in 17-OHCS levels.⁹ Similar secretion depressions have been observed in the steroid levels of hypnotized subjects.^{2,17} Such observations point to the conclusion that the

adrenal cortex responds to emotional events and ordinary experiences. The central nervous system, regulating the adrenalcortical function, involves both elevation and depression of 17-OHCS levels in the body. Therefore, we conclude that the emotional impact of this experiment on the subjects influenced the observed steroid levels.

In a recent review article, von Euler⁷ showed that there is a definite correlation between the urinary excretion of catecholamines and the various types of stress. Generally, it appears that anger, aggression, or exhilaration are associated with increased excretion of norepinephrine; while emotional states characterized by apprehension, discomfort, painful or unpleasant feelings are associated with increased epinephrine excretion.

It was learned from debriefing sessions and diaries that the subjects experienced all the emotional states given above. The interesting part was that these factors seemed to manifest themselves especially on the weekends, when the subjects had an increase of leisure time. Even though the individual curves went in the same direction, they varied since men react differently to different stimuli. While one subject was very stable and showed only small changes under these conditions, another subject showed extreme variability—his values being influenced by numerous factors.

An interesting difference was seen between the restday values for 17-OHCS and corticosterone-like hormones, while the workday values were essentially the same. An explanation for this is that the excretion of the corticosterone-like hormones was more sensitive to psychologic and physiologic stimuli than the 17-OHCS. The values for these variables did not exceed the normal physiological range which could be expected for this type of experiment.

ACKNOWLEDGMENT

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Study of Man During a 56-day Exposure to an Oxygen-Helium Atmosphere at 258 mm. Hg Total Pressure

VII. Respiratory Function

CAPT. W. G. ROBERTSON, USAF, BSC, and A2C. G. L. McRAE

The respiratory effects of a 56-day exposure to an oxygen partial pressure of 175.2 mm. Hg with a helium partial pressure of 73.9 mm. Hg at a total pressure of 257.7 mm. Hg were evaluated in four healthy U. S. Air Force crew members. Control data were obtained during an 8-day pre-experimental and a 4-day postexperimental period. Steady-state carbon monoxide diffusing capacities were unchanged pre- and postexperimentally. Basal oxygen consumption and carbon dioxide production were increased throughout the experimental period. This increase is attributed to an increased water loss at altitude. Vital capacities were decreased approximately 4 per cent with ascent to altitude but returned toward pre-experimental values with time. Vital capacities were all normal immediately on descent from altitude. Lung compartment measurements reflected the decrease in vital capacity at altitude as an apparent decrease in total lung volume as a function of a decreased expiratory reserve volume. Residual volumes were unchanged. Maximum breathing capacities increased approximately 40 per cent as a function of the decreased atmospheric density. There were no changes that would indicate that this atmosphere produces any impairment of man's pulmonary function.

THE RESPIRATORY EFFECTS of chronic exposure to various gaseous atmospheres at hypobaric pressures have been reported by several investigators.^{11,16,17,18} Only one report has dealt with an oxygen-helium atmosphere at decreased pressure.¹⁷ In general the effects noted in these studies were a slight decrease in metabolic rates as a function of confinement and changes in pulmonary function due to the decreased density of the inspired gas mixtures.

The purpose of this study was to evaluate the respiratory effects of a 56-day exposure to an average oxygen partial pressure of 175.2 mm. Hg, helium partial pressure of 73.9 mm. Hg and 257.7 mm. Hg total pressure. Such an atmosphere provides an alveolar oxygen tension of 104 mm. Hg in the presence of helium at the decreased pressure noted. This atmosphere has a density which is equivalent to a 190 mm. Hg pure oxygen atmosphere and the approximately 44 per cent oxygen-

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TABLE I. FREQUENCY OF RESPIRATORY STUDIES

Frequency	2x/7 days	1x/7 days	Pre-Post (in addition to routine baselines)
Measurement	$\dot{V}_E$ $\dot{V}_I$ $\dot{V}_{O_2}$ $\dot{V}_{CO_2}$ Respiratory Rate Vital Capacity Timed Vital Capacity Maximum Breathing Capacity R P_{AO_2} P_{ACO_2}	Lung Compartments	Chest X-Ray Steady-State CO Diffusing Capacity

56 per cent helium atmosphere studied previously. This test represents the longest single experiment in a continuing atmospheric selection program designed to provide man in space with the best environment for a specific mission.

METHODS

Four healthy male volunteer aircrew members were studied. The experimental setting, overall experimental design and the subjects' physical characteristics are presented elsewhere.⁸ Respiratory studies were performed with the frequency indicated in Table I. All measurements were performed by the subjects with the exception of gas analysis, x-rays and carbon monoxide diffusing capacities. Intensive training was provided prior to entry into the chamber to insure that valid data would be obtained.

Since the 4-Man Test Cell provides sleeping accommodations for only two, the subjects were divided into 2-man crews with one crew sleeping from noon to 8:00 p.m. and the other crew sleeping from midnight to 8:00 a.m. Respiratory studies were performed following sleep periods with the crewmen who had been awake performing the tests. Tests were accomplished during the entire 68 days the subjects lived in the chamber. This was divided into an 8-day pre-experimental period, a 56-day experimental period and a 4-day postexperimental period. The respiratory atmosphere was air during the pre- and postexperimental periods at ground-level pressure (747 mm. Hg) and an oxygen-helium mixture at ~258 mm. Hg total pressure during the experimental phase.⁸

Carbon monoxide diffusing capacities were measured immediately before the oxygen-helium exposure and repeated within one hour of the subjects being returned to ground-level pressure following the experimental period. Resting diffusing capacities were measured by the steady-state method of Filley.⁵ The inspired gas mixture for this test was 871 parts per million carbon monoxide in air.

Basal metabolic rates were measured with the subjects fasting and recumbent immediately after they awakened from sleep. The subjects inspired from the chamber atmosphere through a set of 1-way Douglas valves and expired into a Douglas bag. A miniature

differential pressure transducer (Statham ± 2.5 psid) was attached to the vestibule of the 1-way valve and used to indicate the pressure changes corresponding to respiratory movements. The output of the transducer was recorded on a Sanborn 158 and the tracing used to calculate the total time of sampling and the number of breaths taken. Sampling time was normally 4 minutes. The volume of the exhaled air in the Douglas bag was measured in a Tissot spirometer. Alveolar air was collected by the Rahn end tidal sampling technique¹⁵ with the subject recumbent immediately following the basal metabolic rate studies. Samples of alveolar air, expired air and the cabin atmosphere were collected in 100 ml. syringes fitted with 3-way stopcocks and lubricated with saturated lithium chloride, transferred from the chamber and analyzed. Oxygen content was measured with a rapid response Beckman E-2 paramagnetic oxygen analyzer and carbon dioxide determined with a Beckman LB-1 infrared CO₂ analyzer on a static sample measurement basis. In order to guarantee the accuracy of gas analysis, calibration gases were used which contained an appropriate background of helium. In addition, calibration gases were used which corresponded to calculated percentages of unknown gases to reduce analytical error. From the values obtained, the expiratory minute volume ($\dot{V}_E$) was calculated for body temperatures, pressure and saturated with water vapor (BTPS). The inspired minute volume ($\dot{V}_I$), oxygen consumption ($\dot{V}_{O_2}$), carbon dioxide production ($\dot{V}_{CO_2}$), respiratory gas exchange ratios (R) and alveolar gas tensions were calculated by the equations presented by Consolazio⁷ and Rahn.¹³

Forced vital capacities, maximum breathing capacity, lung volumes and maximum expiratory air flows were measured with a Servo-Spirometer, Model 350, made by Med-Science Electronics with the output recorded on a Sanborn 158. To perform a vital capacity, the spirometer was filled to 4.5 liters with chamber air, the subject was instructed to put on a nose clip, expire to normal end expiration and to take 2 or 3 normal breaths from the spirometer before performing a classic forced vital capacity. These records could then be used to determine forced vital capacity, the one- and two-second vital capacity, expiratory flow rate, tidal volume, expiratory reserve and inspiratory reserve volumes. Residual volumes were determined by the 3-breath method of Rahn.¹⁴ Total lung volume was calculated as the sum of the residual volume and the forced vital capacity. Maximum breathing capacities were determined with the subject wearing a nose clip and breathing on the spirometer for 15 seconds at his own selected rate and depth of breathing.

RESULTS

The alveolar oxygen and carbon dioxide tensions for each phase of the experiment are shown in Table II. The high alveolar oxygen tensions measured in the pre-experimental phase are a result of a calibration error in the onstream chamber oxygen control system in addition to the hyperventilation indicated by the low

TABLE III.

Subject No.
66
67
68
69

TABLE

Subject No.	Pre	Exp	Post
66			
67			
68			
69			

Subject No.	Pre	Exp	Post
66			
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68			
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Subject No.
66
67
68
69

STUDY OF MAN IN OXYGEN-HELIUM ATMOSPHERE—VII. RESPIRATORY FUNCTION—ROBERTSON & McRAE

TABLE II. ALVEOLAR GAS TENSION

Subject No.	Pre-Experimental		Experimental		Postexperimental	
	PAO ₂	PACO ₂	PAO ₂	PACO ₂	PAO ₂	PACO ₂
66	112.9 ± 5.8	33.9 ± 2.0	105.0 ± 2.2	38.9 ± 1.6	99.3 ± 4.7	38.7 ± 0.9
67	104.3 ± 2.6	39.7 ± 2.2	100.2 ± 2.6	40.3 ± 1.5	95.9 ± 2.2	39.5 ± 2.7
68	116.1 ± 3.7	38.6 ± 0.5	102.5 ± 3.0	39.6 ± 1.4	100.7 ± 2.9	40.4 ± 0.6
69	109.9 ± 2.8	34.1 ± 0.7	105.7 ± 2.2	38.5 ± 2.1	101.6 ± 4.0	36.4 ± 1.5

TABLE III. CARBON MONOXIDE DIFFUSING CAPACITY (ml/min/mm.Hg)

Subject No.	Pre-Experimental	Postexperimental
66	20.3	18.7
67	14.8	16.5
68	16.7	17.4
69	12.9	12.6

TABLE IV. VENTILATION DURING BMR STUDIES

Subject No.		$\dot{V}_E$ l/min _{BTPS}	Respiratory Rate Breaths/Min	Tidal Volume ml _{BTPS}
66	Pre	8.06 ± 0.39	10.8 ± 1.8	747 ± 67.0
	Exp	7.85 ± 0.68	10.9 ± 1.8	739 ± 43.0
	Post	5.44 ± 0.66	10.6 ± 0.8	511 ± 30.0
67	Pre	6.10 ± 0.74	8.6 ± 1.2	722 ± 58.0
	Exp	6.07 ± 1.11	8.6 ± 1.1	706 ± 31.0
	Post	5.11 ± 0.35	9.1 ± 1.2	569 ± 89.0
68	Pre	6.48 ± 0.63	12.4 ± 1.8	529 ± 69.0
	Exp	6.85 ± 0.62	12.3 ± 1.3	561 ± 74.0
	Post	6.55 ± 0.83	12.5 ± 0.9	524 ± 50.0
69	Pre	9.62 ± 1.29	10.6 ± 1.2	927 ± 71.0
	Exp	6.68 ± 0.74	9.8 ± 1.4	693 ± 35.0
	Post	7.20 ± 0.63	9.9 ± 1.3	751 ± 16.2

TABLE V. METABOLIC RATES

Subject No.		$\dot{V}_{O_2}$ ml/min _{STPD}	$\dot{V}_{CO_2}$ ml/min _{STPD}	R
66	Pre	256.4 ± 27.8	219.3 ± 8.7	0.861 ± .07
	Exp	287.8 ± 54.7	232.8 ± 29.8	0.831 ± .07
	Post	214.3 ± 20.9	172.9 ± 13.5	0.808 ± .02
67	Pre	234.5 ± 50.4	186.7 ± 22.3	0.812 ± .02
	Exp	282.2 ± 48.9	198.9 ± 20.9	0.724 ± .07
	Post	210.3 ± 18.9	169.7 ± 13.4	0.809 ± .06
68	Pre	245.5 ± 43.2	194.7 ± 23.5	0.806 ± .02
	Exp	298.8 ± 46.1	210.1 ± 78.9	0.719 ± .01
	Post	255.3 ± 20.4	199.6 ± 16.1	0.780 ± .09
69	Pre	342.8 ± 69.3	254.4 ± 50.6	0.742 ± .03
	Exp	293.1 ± 17.1	201.2 ± 52.6	0.711 ± .05
	Post	275.7 ± 4.0	226.2 ± 8.0	0.787 ± .06

TABLE VI. VITAL CAPACITY (ml_{BTPS})

Subject No.	Pre-Experimental	Experimental	Postexperimental
66	4704 ± 82	4525 ± 75	4850 ± 80
67	5575 ± 96	5312 ± 146	5449 ± 84
68	5865 ± 32	5693 ± 253	5983 ± 138
69	5257 ± 86	5117 ± 172	5188 ± 142

TABLE VII. MEAN PEAK EXPIRATORY FLOW DURING FORCED VITAL CAPACITY (l/sec)

Subject No.	Pre-Experimental	Experimental	Postexperimental
66	7.1 ± 0.9	11.2 ± 0.9	10.3 ± 0.8
67	11.5 ± 0.5	14.8 ± 1.7	11.9 ± 0.3
68	8.1 ± 1.1	14.6 ± 2.4	11.8 ± 0.4
69	9.8 ± 0.5	12.9 ± 0.8	10.1 ± 0.3

alveolar carbon dioxide tensions in subjects 66 and 69. The values obtained during the experimental phase indicate the excellent control of the chamber atmosphere during the helium phase of the test and that the alveolar gas tensions were normal for this period.

Carbon monoxide diffusing capacities are shown in Table III. There were no changes in diffusing capacity noted for any subject and all values fall within the range of normal values. The values are slightly lower than one would normally expect but are a result of using 871 parts per million carbon monoxide instead of 1000 parts per million carbon monoxide in air as the inspired mixture. Chest x-rays were normal pre- and postexperimentally.

Expiratory minute volumes ($\dot{V}_E$), respiratory rates and tidal volumes observed during basal metabolic rate studies are given in Table IV. The hyperventilation indicated in the alveolar gas measurements for subjects 66 and 69 for the pre-experimental period is also apparent in the $\dot{V}_E$ values. Subjects 66 and 67 had a significantly decreased $\dot{V}_E$ postexperimentally. Both subjects reported a subjective difference in breathing on the Douglas valve on returning to the higher density ground-level atmosphere. This difference was described as an increased resistance in the system.

Oxygen consumption and carbon dioxide production rates (Table V) were significantly increased during the oxygen-helium exposure in three of the four subjects. There were no differences pre- and postexperimentally in the same three subjects. The fourth subject (subject 69) had trouble sleeping during the early phases of the test and this is reflected in the high basal metabolic rates shown in the pre-experimental period. Respiratory exchange ratios (R) were not significantly different throughout the test.

Forced vital capacities are given in Table VI. Vital capacities were decreased by approximately 4 per cent immediately on ascent to altitude and returned toward normal in all subjects. Subjects 68 and 69 produced pre-experimental values by the third week of the altitude phase; subject 67, by the fifth week; and subject 66 never reached pre-experimental values. Even after returning to normal values, subjects 67, 68, and 69 all

TABLE VIII. TIMED VITAL CAPACITY (%)

Subject No.	One (1) Second			Two (2) Seconds		
	Pre-Exp	Exp	Postexp	Pre-Exp	Exp	Postexp
66	80.7 ± 1.9	88.0 ± 4.0	84.4 ± 2.1	93.4 ± 2.0	95.7 ± 2.4	95.8 ± .5
67	84.9 ± .8	88.8 ± 2.4	86.4 ± .9	96.6 ± 1.8	96.8 ± 1.9	98.1 ± 2.3
68	84.3 ± .5	87.5 ± .6	83.6 ± 1.3	96.5 ± 4.1	95.7 ± 2.3	95.2 ± .6
69	79.5 ± 5.9	90.5 ± 1.7	80.7 ± 2.8	94.2 ± 2.7	97.3 ± 1.2	92.7 ± 1.7

showed decreased vital capacities on some occasions.

Peak expiratory flows (Table VII) produced during the forced vital capacity maneuvers were increased during the experimental period. Postexperimentally flows were higher than during the experimental period. This is believed to be an indication of thoracic muscle conditioning due to the frequency of producing high ventilatory rates during exercise periods²⁰ and pulmonary function testing.

One-second timed vital capacities (Table VIII) were increased during the experimental phase. Two-second values showed no differences.

Maximum breathing capacities (Table IX) were increased immediately upon entering the oxygen-helium atmosphere at decreased pressure and remained remarkably constant for the entire 56 days. Upon return to ground level, values were slightly increased in subjects 66 and 69. Since the standard deviations for these subjects were so small during the experimental phase, the postflight increase cannot be attributed to a learning effect, but rather to a muscle training effect. The decrease noted for subjects 67 and 68 postexperimentally resulted from a change in the pattern with which they performed the test. Both subjects changed their pattern to a short, fast delivery which decreased their ability to move air. This is also indicated in their mean peak expiratory flow rates (Table X) postexperimentally.

Residual volumes (Table XI) were the same throughout all phases of the test. Tidal volumes and inspiratory reserve volumes were also unchanged. Expiratory reserve volumes were decreased and resulted in the decrease in vital capacity noted above and in an apparent decrease in total lung volume since it was calculated as the sum of the residual volume and the vital capacity. The changes described as time course changes in vital capacities are all a result of changes in the expiratory reserve volume.

DISCUSSION

As compared to previous atmospheric tests,^{11,16,17,18} there was one major design difference included in this test. A special effort was made to guarantee physical conditioning and to prevent cardiovascular deconditioning or a hypodynamic response to confinement. The exercise program used proved adequate to prevent such a response.²⁰

The metabolic rates measured during this test are in direct contrast to any data obtained previously. In past tests oxygen consumptions have either not changed¹⁷ or decreased continually over the period of exposure.^{16,18} The difference between the tests is the

TABLE IX. MAXIMUM BREATHING CAPACITIES (l/min_{BTPS})

Subject No.	Pre-Experimental	Experimental	Postexperimental
66	179.0 ± 0.8	270.8 ± 5.5	192.5 ± 8.5
67	193.5 ± 34.4	249.0 ± 34.2	161.8 ± 22.2
68	203.7 ± 14.5	321.8 ± 6.1	181.2 ± 15.1
69	175.2 ± 7.7	278.9 ± 16.1	197.4 ± 21.2

TABLE X. MEAN PEAK EXPIRATORY FLOW RATE DURING MBC (l/sec)

Subject No.	Pre-Experimental	Experimental	Postexperimental
66	7.4 ± 0.4	12.6 ± 2.1	10.1 ± 0.6
67	9.5 ± 0.6	10.7 ± 0.5	7.7 ± 2.8
68	9.0 ± 0.4	13.0 ± 1.7	8.8 ± 0.4
69	9.6 ± 0.7	13.3 ± 2.0	10.3 ± 0.6

TABLE XI. RESIDUAL VOLUME (ml_{BTPS})

Subject No.	Pre-Experimental	Experimental	Postexperimental
66	1035 ± 98	1078 ± 126	1065 ± 88
67	1249 ± 63	1236 ± 94	1256 ± 58
68	1610 ± 77	1596 ± 115	1585 ± 84
69	1351 ± 55	1375 ± 83	1363 ± 63

absence of hypokinesia in the current experiment. The increase in oxygen consumptions measured in this test is not fully explained. Accelerated metabolism has been reported on mice^{2,3,9} and men¹² as a function of an increased heat loss in the presence of helium. However, a theoretical analysis of the thermal exchange of this atmosphere has been performed by Epperson, et al.⁴ that indicates that heat transfer in this atmosphere is not very different from air at ground level and would probably not have a measurable effect. Water balance studies, carried out during this study and reported by Glatte,⁶ showed an increase in water loss at altitude. An increased insensible weight (water) loss at altitude has been described by McCutchan,¹⁰ Hale, et al.,⁷ and Webb, et al.,¹⁹ as function of increased diffusion as the barometric pressure is lowered. If insensible water loss is indeed elevated at decreased barometric pressure, then this must be an obligatory loss and mandate an increased oxygen consumption to provide the necessary heat of vaporization. The magnitude of increased water loss measured in this test can completely explain the increased oxygen requirement measured.

Changes in maximum breathing capacities and flow rates are of the order of magnitude expected for an atmosphere with this density. The results are similar to those reported by Ulvedal¹⁸ at 190 mm. Hg total pressure with pure oxygen and Robertson¹⁷ at 380 mm. Hg total pressure with approximately 44 per cent oxygen-56 per cent helium atmosphere as the inspired mixture. These three atmospheres are essentially equivalent density atmospheres. The increases noted result from a reduction in resistance to respiratory gas flow as a function of the decreased density.

Changes in forced vital capacities have been reported previously,^{16,17,18} but an exact mechanism for this effect has not been delineated. Several possible causes have been suggested.¹⁴ Assuming the thoracic muscles have the same capacity for applying force across the lung at altitude, it is suggested that the increased compressibility of the gases, due to decreased molecular abundance, coupled with patency of airways, results in gas trapping at decreased barometric pressure and, therefore, the observed decreases in vital capacities. Unpublished data obtained in this laboratory tend to indicate that this hypothesis is valid, but further tests are needed. The increased flow rates and one-minute vital capacities are a direct resultant of the decreased airway resistance and increased laminar air flow as a function of decreased atmospheric density.

From these findings there is no contraindication that this atmosphere is in any way limiting to respiratory function in man.

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Study of Man During a 56-day Exposure to an Oxygen-Helium Atmosphere at 258 mm. Hg Total Pressure

VIII. Observations on Feeding Bite-size Foods

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Twenty-one bite-size foods supplemented with eight fruit juices, an enriched drink, coffee, tea, sugar, a vitamin-mineral tablet and water ad libitum were arranged into a 2-day menu cycle. This feeding system was offered to 4 flight crew members as the sole source of nutrients for a period of 72 days, including 56 consecutive days in a space vehicle simulator containing an atmosphere of 257.7 mm. Hg total pressure including 175.2 mm. Hg P_{O_2} and 73.9 mm. Hg P_{He} .

The relationship between the consumption of food and the following was studied: initial and mean acceptability ratings by the subjects, ratings by a technical taste panel, rehydratability, and fat content of the food. Trends in each subject's consumption and acceptability rating of each food item were studied as a function of time.

The manufacturing procedures and criteria for each food are outlined. Suggestions are offered for adapting this type of feeding system to aerospace situations.

ease of handling, and minimum rehydration time.

This paper describes a feeding system based primarily on bite-size foods which meet many of the criteria for use in space vehicles. This feeding system was provided for 4 subjects during a period of 72 days in support of a comprehensive study to evaluate an $O_2:He$ atmosphere. Details of the design of the overall study are reported elsewhere.⁸

A feeding system based on bite-size foods was selected for this study because if such a system could be improved and adapted for use in space vehicles it would be a relatively simple system, since it offers high caloric density and would minimize problems associated with rehydrating food during weightlessness, measuring consumption, and disposing of wet residue.

MATERIALS AND METHODS

Twenty-one bite-size foods, eight fruit juices, an enriched drink, coffee, tea, sugar, and a vitamin-mineral tablet were arranged in a 2-day menu cycle consisting of 4 meals per day as shown in Table I. Water was provided ad libitum. Figures 1 through 6 show the bite-size foods as they occurred in the menu.

This feeding system was provided for 4 subjects for a period of 72 days, including 68 consecutive days in a 4-man space cabin simulator² of which 56 consecutive days consisted of exposure to 257.7 mm. Hg total pressure including 175.2 mm. Hg P_{O_2} and 73.9 mm. Hg P_{He} . The 4 subjects consisted of 2 USAF copilots and 2 navigators. Their ages ranged from 27 to 29 years. Additional information on the subjects is reported elsewhere.⁸

The weight of each portion of food provided was recorded to the nearest hundredth of a gram. The bite-size food components of each meal were placed in a polyethylene bag and the fruit juice and enriched drink powders were placed in 480 ml. Mason-type jars for ease of reconstitution by the subjects prior to consumption. The food was given to the subjects in exchange for unconsumed food from the previous day. The unconsumed food was weighed and the quantity of consumed food calculated by difference. Each subject was given a food evaluation form each day soliciting an acceptance rating for each food on the 9-point hedonic scale originally designed to measure food preferences of large groups.¹⁴ The rating forms were collected at

THE PROVISION of an adequate feeding system is one of the critical problems of life support in manned space missions. Many types of in-flight aerospace feeding systems have been suggested.^{3,7,10,12,19,22} Several of these systems have been evaluated under a variety of conditions.^{6,9,11,13,15,17,18}

Technical constraints imposed on food by space vehicle environments may be classified as biological, engineering, and operational. The biological constraints include considerations for food safety, acceptability, gastroenterology, nutrition, and dietetics. The engineering constraints are determined by weight and volume limitations and requirements for tolerance to pressure differentials, shock, acceleration, relative humidity, and vibration as well as the limits imposed by the characteristics of the water available for rehydration. The operational constraints include requirements for heating, cooling, residue disposal, vehicle interface mating, usability at zero g, freedom from crumbling,

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the end of each day. The subjects were also invited to make any written comments concerning the food.

The production procedures and ingredients of the bite-size foods and fruit juices are summarized in Table II. The enriched drink was manufactured in powder form for reconstitution with two parts of water by weight. The formulation of this powder mix was: dry powdered skim milk 40 gm., sucrose 20 gm., lac-

tose 20 gm., dextrose and maltose* 100 gm., coffee whitener† 100 gm., sodium caseinate 17.5 gm., sodium chloride 1.5 gm., and magnesium oxide 0.1 gm.

Initial calculations of the nutritional content of this feeding system were based on proximate analysis of

*Dextri-Maltose Formula #1, Mead Johnson & Co.
†"Coffee-mate," Carnation Co.

TABLE I. MENU AND AVERAGE WEIGHT OF EACH PIECE OF FOOD

Day 1	Day 2
<i>Meal A</i>	<i>Meal E</i>
4 Bacon squares (22.3 gm.)	4 Bacon squares (22.3 gm.)
6 Chicken sandwiches (39.2 gm.)	6 Cheese sandwiches (38.2 gm.)
6 Gingerbread bites (43.0 gm.)	6 Strawberry cereal cubes (36.2 gm.)
6 Peanut cubes (47.9 gm.)	6 Brownies (43.4 gm.)
1 Beverage*	1 Beverage*
<i>Meal B</i>	<i>Meal F</i>
6 Beef bites (31.8 gm.)	6 Bacon and egg bites (37.3 gm.)
6 Apricot cubes (52.0 gm.)	6 Toasted bread cubes (36.0 gm.)
4 Date fruitcakes (54.3 gm.)	6 Pineapple cubes (54.1 gm.)
6 Cinnamon toasts (21.0 gm.)	6 Potato chip cubes (30.0 gm.)
1 Beverage*	1 Beverage*
<i>Meal C</i>	<i>Meal G</i>
1 Enriched drink†	1 Enriched drink†
1 Vitamin-trace mineral tablet‡	1 Vitamin-trace mineral tablet‡
<i>Meal D</i>	<i>Meal H</i>
6 Beef sandwiches (45.3 gm.)	6 Chicken bites (27.2 gm.)
4 Pineapple fruitcakes (55.0 gm.)	6 Toasted bread cubes (36.0 gm.)
6 Peanut cubes (48.0 gm.)	6 Apricot cereal cubes (38.4 gm.)
6 Chocolate ice cream cubes (33.3 gm.)	6 Peanut cubes (47.9 gm.)
1 Beverage*	4 Candy cubes (34.5 gm.)
	1 Beverage*

*Beverage was free choice of one from the following group (each reconstituted with approximately 360 ml. of water):

Orange (42 gm.)	Pineapple (56 gm.)
Grapefruit (42 gm.)	Orange-grapefruit (42 gm.)
Apple (50 gm.)	Orange-pineapple (49 gm.)
Grape (54 gm.)	Pineapple-grapefruit (49 gm.)

†Quantity of enriched drink was varied per individual on the basis of lean body mass.

Subject #66	60.0 gm.	Subject #68	100.0 gm.
Subject #67	104.0 gm.	Subject #69	54.0 gm.

‡Vitamin-trace mineral tablet contained the following:

Vitamin A	1.50 mg.	Iron (as sulfate)	10.00 mg.
Vitamin D	10.00 µg.	Copper (as sulfate)	1.00 mg.
Thiamin mononitrate	3.00 mg.	Calcium (as iodate)	0.15 mg.
Riboflavin	2.50 mg.	Cobalt (as sulfate)	0.10 mg.
Nicotinamide	20.00 mg.	Manganese (as sulfate)	1.00 mg.
Pyridoxine hydrochloride	0.50 mg.	Magnesium (as oxide)	5.00 mg.
Cobalamin	2.00 µg.	Zinc (as sulfate)	1.50 mg.
Calcium pantothenate	5.00 mg.	Molybdenum (as sodium molybdate)	0.20 mg.
Ascorbic acid	50.00 mg.		

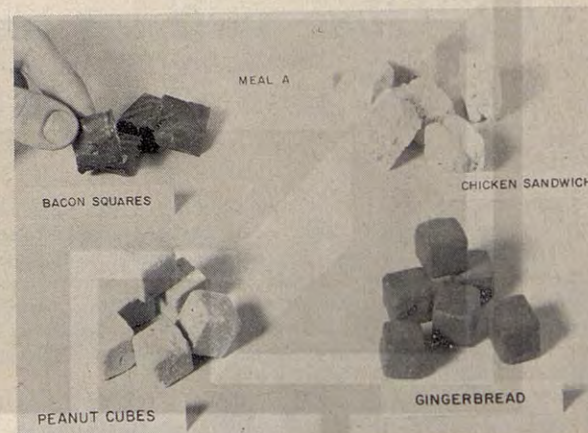


Fig. 1. Bite-size foods offered in first meal on odd numbered days.

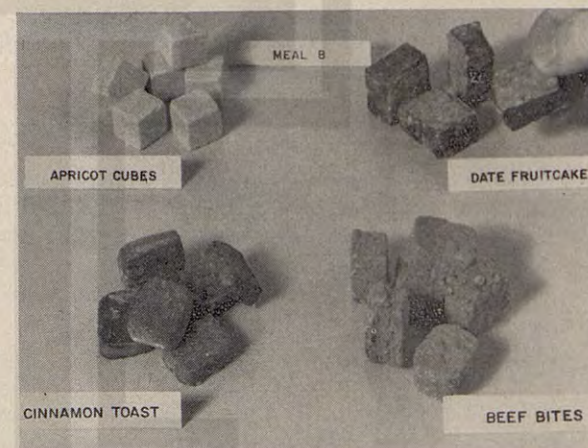


Fig. 2. Bite-size foods offered in second meal on odd numbered days.

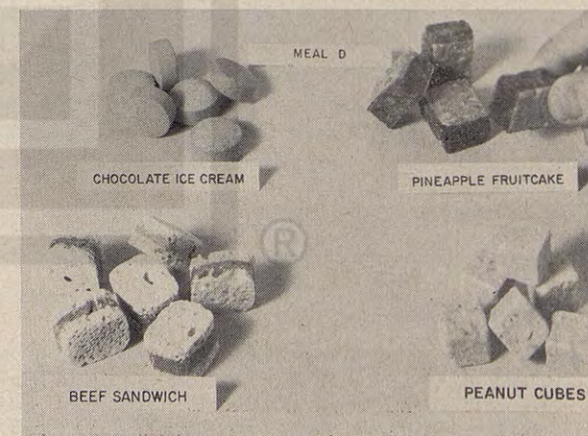


Fig. 3. Bite-size foods offered in fourth meal on odd numbered days.

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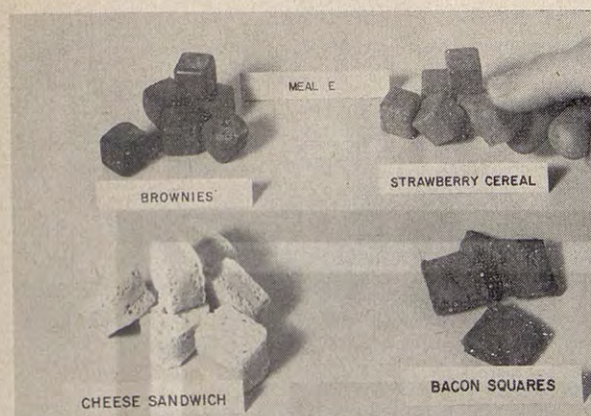


Fig. 4. Bite-size foods offered in first meal on even numbered days.

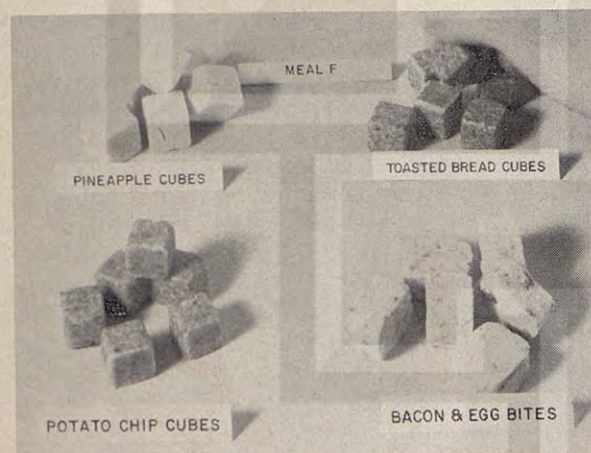


Fig. 5. Bite-size foods offered in second meal on even numbered days.

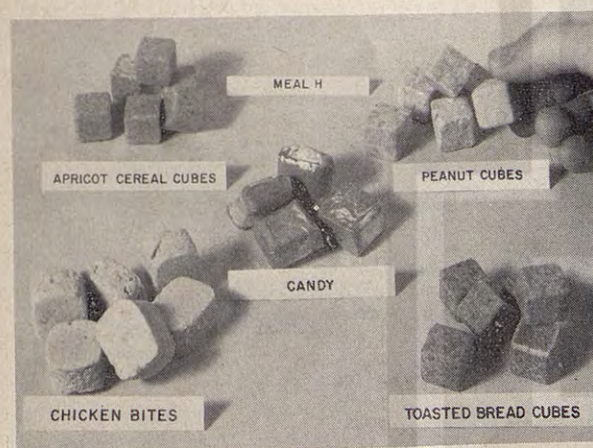


Fig. 6. Bite-size foods offered in fourth meal on even numbered days.

TABLE II. OUTLINE OF FOOD PRODUCTION TECHNIQS

Food item	Principal ingredients	Sequence of processing procedures	Coating
Apricot cereal cube	Corn flakes, shortening, apricots, egg albumin, sorbitol	Mix, compress, freeze-dry, coat	Gelatin

Apricot cube	Fat, milk solids, sugar, apricots	Mix, mold, coat	Zein
Bacon & egg bite	Eggs, milk solids, bacon, shortening	Cook, cut, freeze-dry, coat	Hydrogenated palm oil
Bacon square	Bacon	Fry, compress, cut	None
Beef bite	Water, beef, gravy mix, shortening	Cook, cut, freeze-dry, coat	Hydrogenated palm oil
Beef sandwich	Beef, bread, beef broth, gravy, water, shortening	Soak in gelatin, cut, freeze-dry, coat	Gelatin and hydrogenated palm oil
Brownie	Sugar, pecans, shortening, eggs, flour, cocoa	Mix, bake, crumb, compress, coat	Protein and fat
Candy	Milk solids, sodium caseinate, sugar, fat	Mix, cook, mold	None
Cheese sandwich	Cheese, bread, gelatin	Soak in gelatin, cut, freeze-dry, coat	Gelatin and hydrogenated palm oil
Chicken bite	Water, chicken, gravy mix, shortening	Cook, cut, freeze-dry, coat	Hydrogenated palm oil
Chicken sandwich	Chicken, bread, gravy mix, gelatin, shortening	Soak in gelatin, cut, freeze-dry, coat	Gelatin and hydrogenated palm oil
Chocolate ice cream	Liquid ice cream mix	Freeze-dry, powder, compress	None
Cinnamon toast	Bread, cinnamon	Toast, soak in gelatin, cut, freeze-dry, coat	Gelatin and hydrogenated palm oil
Date fruitcake	Dates, pecans, eggs, cherries, sugar, flour	Mix, bake, cut, freeze-dry, coat	Gelatin and starch
Fruit juice	Natural juices	Concentrate, dry	None
Gingerbread	Molasses, flour, sugar, shortening, water, eggs	Mix, bake, crumb, compress, coat	Protein and fat
Peanut cube	Fat, milk solids, sugar, peanuts	Mix, mold, coat	Zein
Pineapple cube	Fat, milk solids, sugar, pineapple	Mix, mold, coat	Zein
Pineapple fruitcake	Pineapple, pecans, eggs, cherries, sugar, flour	Mix, bake, cut, freeze-dry, coat	Gelatin and hydrogenated palm oil
Potato chip cube	Potato sticks, crackers, sorbitol	Grind, mix, blend, compress, freeze-dry	None
Strawberry cereal cube	Corn flakes, shortening, strawberries, egg albumin, sorbitol	Mix, compress, freeze-dry, coat	Gelatin
Toasted bread cube	Toast crumbs, shortening, egg albumin, malt syrup, sucrose, sorbitol	Mix, press, cut, coat	Gelatin

similarly produced foods and reported food values.²¹ The feeding system was thus designed to provide 45 kilocalories per kilogram of lean body weight¹ plus 600 kilocalories for exercise. The exact quantities of nutrients actually provided by this feeding system, as determined by chemical analysis, are reported in the following paper.²⁰ The adjustment of nutrient content to meet each subject's individual requirement was obtained by adjusting the quantity of enriched drink provided to each subject.

The rehydratability of each bite-size food item was determined as the percentage weight gained by the item following 5 minutes' submersion in 37°C. human saliva. The acceptability rating of each bite-size food was determined by a technical taste panel[‡] composed of an average of 8 members. The fat content of each food item was obtained by chloroform-methanol extraction.²⁰

Using the least squares criterion,¹⁶ a straight line of "best" fit was computed for the relationship between each subject's consumption of each bite-size food (y)

†Furnished by the U. S. Army Natick Laboratories.

TABLE III. MICROBIOLOGICAL REQUIREMENTS* FOR FOODS USED IN 72-DAY STUDY

Organisms	Required limit
Total aerobic plate count	Not greater than 10,000 per gram
Total coliform count	Not greater than 10 per gram
Fecal coliform count	Negative in 0.5 gram
Fecal streptococci count	Not greater than 20 per gram
Coagulase positive staphylococci	Negative in 0.5 gram
Salmonellae	Negative in 5.0 gram

*When examined by the methods suggested by El-Bisi.⁴

TABLE IV. CONSUMPTION AND ACCEPTABILITY RATINGS* OF BITE-SIZE FOODS BY FOUR SUBJECTS DURING A 72-DAY STUDY

Food item (Subject)	Per cent consumption by each subject				First† acceptability rating of each subject				Mean acceptability ratings of each subject				Range of acceptable ratings of each subject			
	66	67	68	69	66	67	68	69	66	67	68	69	66	67	68	69
Apricot cereal cubes	37	100	96	94	7	7	7	5	7.1	6.6	4.7	7.4	6-8	4-9	3-7	7-8
Apricot cubes	91	55	95	88	7	7	4	6	7.4	4.9	4.5	6.9	7-8	2-7	3-6	6-8
Bacon and egg bites	89	77	63	74	9	9	8	8	6.5	4.1	1.6	6.1	6-8	1-9	1-6	5-7
Bacon squares	100	93	100	92	7	8	5	8	8.0	7.6	5.2	8.2	7-8	6-9	3-7	1-9
Beef bites	89	63	72	65	4	3	1	2	6.1	2.3	1.0	3.9	4-7	1-6	1-2	2-6
Beef sandwiches	33	57	64	23	3	2	1	2	5.6	1.9	1.0	2.7	3-6	1-5	1-1	2-4
Brownies	100	100	100	99	8	9	8	8	8.0	7.1	6.5	7.9	8-8	4-9	5-8	7-8
Candy	25	95	99	97	7	9	8	8	6.1	5.2	5.1	8.0	4-7	2-9	3-8	7-9
Cheese sandwiches	100	64	100	92	7	7	7	5	7.9	4.3	2.4	6.5	7-8	2-7	1-7	5-7
Chicken bites	36	64	69	36	7	4	6	7	6.2	3.3	1.3	3.7	4-7	1-7	1-4	2-6
Chicken sandwiches	97	59	73	43	3	3	1	2	6.1	3.0	1.1	3.4	3-8	1-6	1-2	2-5
Chocolate ice cream	33	95	87	99	8	9	8	8	6.7	5.5	5.1	8.2	6-8	2-9	3-9	7-9
Cinnamon toast	68	97	97	90	7	7	7	7	4.7	6.3	5.4	7.7	4-7	4-9	3-7	7-8
Date fruitcakes	91	97	96	58	8	7	8	7	8.0	6.4	6.5	5.7	8-8	4-9	5-8	4-7
Gingerbread	100	100	100	98	7	7	8	5	7.7	7.2	6.5	7.2	7-8	4-9	5-8	5-8
Peanut cubes	62	98	99	81	7	9	8	7	7.3	5.0	6.5	7.0	7-8	2-9	5-8	6-7
Pineapple cubes	91	50	95	85	7	7	7	6	7.0	4.8	5.0	7.0	7-7	1-8	3-8	7-8
Pineapple fruitcakes	35	99	96	62	8	7	8	7	8.0	6.1	6.6	5.8	8-8	3-9	5-8	4-7
Potato chip cubes	91	03	17	09	7	8	8	7	6.8	1.1	1.5	2.0	6-7	1-4	1-6	1-7
Strawberry cereal cubes	100	100	100	99	7	9	8	8	7.5	7.0	5.2	7.9	6-8	4-9	4-8	7-8
Toasted bread cubes	62	96	87	65	7	7	5	6	7.1	5.9	4.3	6.6	7-8	3-9	3-7	5-8

*Acceptability rating on a 9-point hedonic scale.

†Acceptability rating given the first time subject tasted the food.

and his first acceptability rating of that food (x). A total of 84 x, y points were used in fitting this "regression" line ($y = a + bx$, where a is the intercept and b is the slope). The first rating was considered the independent variable and the percent consumption the dependent variable. Similarly, a straight line of "best" fit was computed for the relationship between each subject's consumption and the mean of all his acceptability ratings of each of those foods. Additional lines were fit for the relationships between consumption of each bite-size food and the following three factors: the rating of the technical taste panel, the rehydratability, and fat content of each food.

The primary purpose of these analyses was to test the hypothesis that consumption could have been predicted from any of the three acceptability ratings, rehydration or fat content of the food. The observed slopes were tested against hypothesized slopes of 0, 6.25, and 12.5 for each of the three sets of data relating consumption to acceptability scores. These hypothetical slopes were selected, since acceptability was measured on a 9-point scale. A positive slope of 12.5 would mean that a rating of 1 (dislike extremely) would correspond to 0 per cent consumption and a rating of 9 (like extremely) would correspond to 100 per cent consumption. A slope of 6.25 would occur when a food item rated at one extreme of the acceptability scale was consumed at a rate 50 per cent different from an item rated at the other end of the scale. Tests were made to determine if the observed slope differed significantly from a hypothesized slope of zero for consumption related to rehydratability and also consumption related to fat content.

Each individual's daily consumption and his daily acceptability rating of each food item were plotted as a function of time. These plots were studied for trends

in consumption and acceptability.

Representative samples of each food were examined for compliance with the microbiological standards shown in Table III.

RESULTS

An overall consumption of 83.8 per cent resulted when a feeding system composed of 21 bite-size foods, 8 fruit juices, an enriched drink, coffee, tea, sugar and a vitamin-mineral tablet arranged into a 2-day menu cycle was offered as the only source of nutrients for 4 Air Force flight crew members for 72 days, including 56 consecutive days exposure to 257.7 mm. Hg total pressure containing 175.2 mm. Hg P_{O_2} and 73.9 mm. Hg P_{He} in a space vehicle simulator. The overall consumption of the bite-size foods was 78.8 per cent. Consumption of individual bite-size items varied from 29 per cent to 99 per cent. The fruit juices and enriched drink were completely consumed. The subjects' mean acceptability ratings of individual bite-size foods, scored on a 9-point hedonic scale, ranged from 2.7 to 7.5.

The subjects were conscientious and cooperative in offering their acceptability ratings and comments concerning the foods during the length of the study. The predominating comment was that the feeding system was essentially a survival ration which prevented hunger, but was very monotonous and afforded little pleasure. The consumption and acceptability ratings given by each subject to the bite-size foods are presented in Table IV. The mean consumption, mean acceptability ratings, rehydratability and fat content of each bite-size food is given in Table V.

The relationship between each subject's consumption

TABLE V. MEAN CONSUMPTION, MEAN ACCEPTABILITY RATING, REHYDRATABILITY, AND PERCENTAGE FAT CONTENT OF BITE-SIZE FOODS OFFERED TO FOUR SUBJECTS DURING A 72-DAY STUDY

Food item	Mean per cent consumption by all subjects during 72 days	Mean of technical taste panel acceptability ratings*	Rehydratability†	Per cent fat‡
Apricot cereal cubes	81	6.9	8.0	16.1
Apricot cubes	82	5.6	3.7	31.0
Bacon and egg bites	75	5.6	11.4	44.4
Bacon squares	96	5.9	25.4	26.6
Beef bites	72	5.3	24.5	42.1
Beef sandwiches	44	4.7	19.1	65.2
Brownies	99	6.5	3.7	18.6
Candy	79	5.6	3.9	23.8
Cheese sandwiches	89	5.5	20.2	46.0
Chicken bites	51	6.4	35.3	40.7
Chicken sandwiches	68	4.6	31.1	50.5
Chocolate ice cream	78	7.0	—	38.1
Cinnamon toast	88	6.7	7.6	40.9
Date fruitcakes	85	7.3	9.8	23.5
Gingerbread	99	6.8	7.0	22.6
Peanut cubes	84	7.9	3.8	32.1
Pineapple cubes	80	6.8	3.3	26.1
Pineapple fruitcakes	73	6.4	4.0	22.3
Potato chip cubes	29	5.9	13.8	24.6
Strawberry cereal cubes	99	6.3	8.6	16.3
Toasted bread cubes	77	6.6	11.2	18.6

*Averaging 8 members.

†Defined as the percentage weight gain following 5 minutes' submersion in human saliva at 37° C.

‡By chloroform-methanol extraction.

of each bite-size food and his first (initial) acceptability rating of that food is presented in Figure 7. The relationship between each subject's consumption of each bite-size food and the mean value of all his acceptability ratings of that food is presented in Figure 8. The relationship between mean value of consumption of each bite-size food by all the subjects and the ratings of each of these foods by a technical taste panel is given in Figure 9. The relationship between the mean value of consumption of each bite-size food by

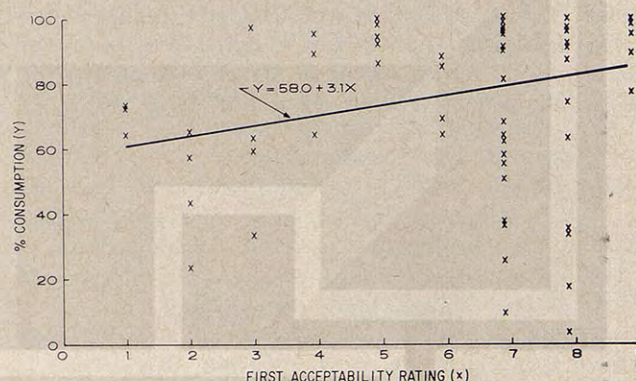


Fig. 7. The relationship between each subject's consumption of each bite-size food and his first (initial) acceptability rating of that food.

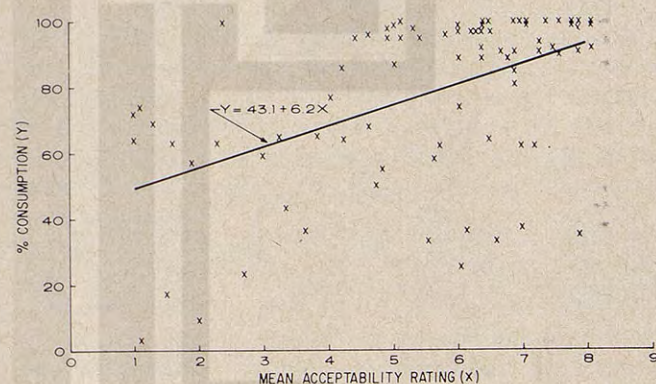


Fig. 8. The relationship between each subject's consumption of each bite-size food and the mean value of all his ratings of that food.

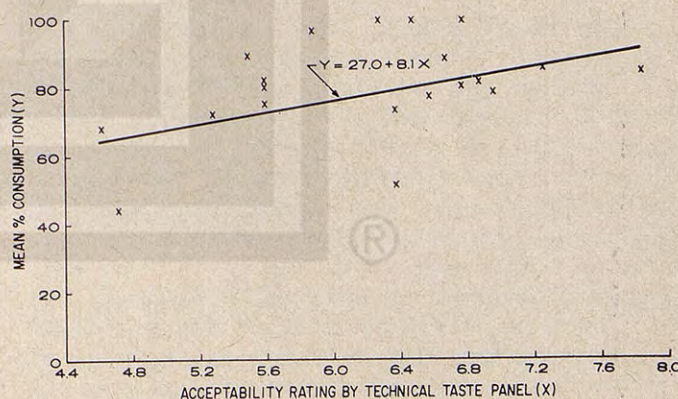


Fig. 9. The relationship between mean value of consumption of each bite-size food by all the subjects and the acceptability ratings of each of these foods by a technical taste panel.

all the subjects and the rehydratability of each of these foods is given in Figure 10. The relationship between the mean value of consumption of each bite-size food by all the subjects and the fat content of each food is given in Figure 11.

The slope for the relationship between each subject's consumption of each bite-size food and his first acceptability rating of that food was significantly different from each of the hypothesized slopes of 0, 6.25, and 12.5 ($P < .05$ in each case). The distribution of these data and the straight line of "best" fit are presented in Figure 7. Similar analyses of the relationship, represented in Figure 8, between each subject's consumption and his mean rating revealed that the slope for this set of data was significantly different from the hypo-

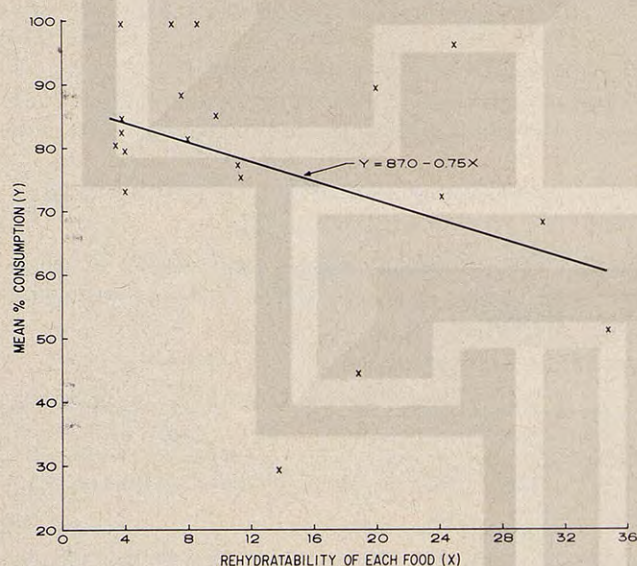


Fig. 10. The relationship between the mean value of consumption of each bite-size food by all the subjects and the rehydratability of each of these foods.

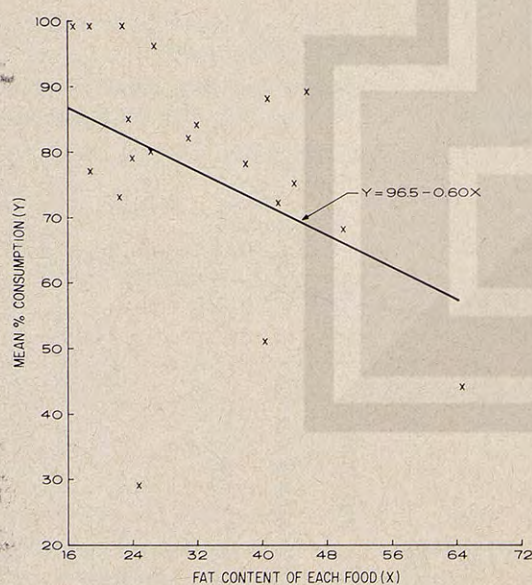


Fig. 11. The relationship between the mean value of consumption of each bite-size food by all the subjects and the fat content of each food.

thesized slopes of 0 and 12.5 ($P < .05$ in each case). This line was not significantly different from the hypothesized line with a slope of 6.25 ($P > .05$).

The line of "best" fit for the relationship between the mean value of the consumption of each bite-size food by all the subjects and the acceptability ratings by the technical taste panel, which is presented in Figure 9, was not found to have a slope significantly different from any of the three hypothesized slopes of 0, 6.25, and 12.5 ($P > .05$ in each case).

The slopes for the relationships between mean consumption and rehydratability and mean consumption and fat content of the food were significantly different from hypothesized slopes of zero. In both of these cases the line of "best" fit exhibited a negative slope.

Inspection of the 42 plots of each subject's daily consumption and acceptability rating of each bite-size food as a function of time revealed several trends. The two subjects (Nos. 66 and 69), on the night work shift, tended to persist in rating each food near their initial rating of that food. The other two subjects who worked on the daytime shift, tended to lower their acceptance rating with time. Consumption of most food items tended to remain at a constant level. Consumption of the following items decreased markedly with time: Potato chip cubes, chicken sandwiches, beef bites, beef sandwiches, and chicken bites. One subject's consumption was erratic, since he consumed meals on an all-or-none basis.

Microbiological examinations showed the foods met the standards shown in Table III. The bite-size foods were highly stable and essentially free from organoleptically detectable oxidation, off-color, off-flavor, stickiness, fat migration, and crumbling.

The feeding system proved convenient for use under the conditions of the study. Operations involved in food preparation, food handling, portion control, recording amount consumed, and waste disposal were minimized by the nature of the bite-size foods. From 30 to 35 minutes was required for eating each bite-size meal.

DISCUSSION

A feeding system based primarily on low moisture content bite-size foods is a very simple and reliable high caloric density feeding system which minimizes problems associated with food rehydration, food heating, portion control, measurement of consumption, and wet residue disposal. A 2-day menu cycle containing a very limited variety of bite-size foods proved satisfactory when consumed by a highly motivated crew under the conditions of this study.

The feasibility of a bite-size feeding system was demonstrated even though the system employed was, in effect, a survival ration containing some poorly accepted food items. This suggests that improvement of such a system by the addition of more variety and more acceptable foods should increase its usefulness. It is suggested that if the number of bite-size foods were increased to approximately 100, then an adequate 6-day, or longer, menu cycle could be designed. The

new food items might contain various levels of moisture in order to provide a variety of mouth feel and texture. Such items could also prove useful in snack-type feeding. With good quality control, it would be possible to fabricate such foods with a high degree of reliability. A feeding system of this type should prove useful in long endurance aerospace flights and military operations.

It was shown that a menu containing a very limited number of food items could be adapted to individual nutritional needs by adjusting the quantity of one food item which contained the correct proportion of all the macronutrients known to be required. In this study, the food was provided in the form of a powder which, when reconstituted with water, made a milk shake-like drink. Many forms and flavors of such foods could be developed, including cakes, candies, and a wide variety of compressed items composed of natural foods or selected highly purified food components, such as isolated proteins, fats, starches, and sugars. The term "macronutrient-balanced food" is proposed for this type of item. Macronutrient-balanced foods also promise potential value for feeding in contingency situations.

The 9-point hedonic scale used in acceptability scoring would have greatest usefulness if a difference in one rating point on the scale corresponded to a difference of 12.5 per cent in consumption (slope of 12.5). This would mean that a rating of 1 (dislike extremely) would correspond to a 0 per cent consumption and a rating of 9 (like extremely) would correspond to 100 per cent consumption. The usefulness of acceptability scoring for predicting consumption decreases as the slope of the relationship between the acceptance scores (measured on a 9-point scale) and the per cent consumption decreases below 12.5.

The slope of the straight line of "best" fit for the relationship between consumption and first acceptability score in this study was 3.1 and it was significantly different ($P < .05$) from hypothetical slopes of 0, 6.25, or 12.5. The first acceptability rating by these subjects was, therefore, not a strong prediction of consumption. However, a moderate correlation did exist between mean score and consumption (slope not significantly different from 6.25). Since the mean score could only have been known after the conclusion of the study, its value for "predicting" consumption is theoretical.

The subjects in this study commented that they would not again be willing to subsist solely on this feeding system unless it were an operational necessity. The tolerance of these subjects to this feeding system was fatigued by the study so that their consumption in any subsequent study could not necessarily be predicted by their responses in this one. This is considered an observation of importance to any proposed study which might involve candidates for future space crews.

Failure of the slope of the relationship between mean consumption and the technical taste panel rating to differ significantly from hypothesized slopes of 0, 6.25, and 12.5 is a function of the scatter of the data. This

taste panel had been used in screening prototypes of these foods during their development and, hence, in general, had previously eliminated foods rated below 6.0.

These impressions must be interpreted within the limits of the study and the statistical test applied. In regression analysis it is assumed that the independent variable (x axis) is measured with negligible error. When the subject's first (initial) acceptability ratings were obtained, the subjects had had no experience in the rating of foods. The last of the 21 foods was, therefore, likely rated in comparison to the first few foods. Consequently, not all of the first ratings were determined exactly or with equal accuracy. Another assumption necessary for valid statistical analysis is normality (symmetrical distribution of the data). Since in this study the consumption could not exceed 100 per cent, the distribution of y values (consumption) was necessarily truncated at 100 per cent.

Another observation concerning these data, as shown in Figure 7, is that two of the bite-size foods which were rated 8 (like very much) and one which was rated 7 (like moderately) experienced a consumption of less than 20 per cent. Such an observation by itself raises doubts concerning the ability of this test method to predict an individual's consumption of food items. Notice that on each of the graphs there is considerable scatter of the data points about the line of "best" fit.

The "prediction" of the regression line in Figure 7 is that food which is rated 1 (dislike extremely) at first tasting would be expected to have experienced 61 per cent consumption and food that received a rating of 9 (like extremely) would have been 86 per cent consumed. Such an unrealistic prediction could be the result of any or all of the factors discussed above, as well as that there may be no relationship between first acceptability ratings and consumption. In addition, this "prediction" could have resulted because the subjects had no other food to eat during the study.

Different eating habits of the individual subjects also weighed heavily upon the distribution of consumption among the bite-size foods. Subject No. 66, for example, was taught in childhood to "clean his plate." This training expresses itself in an all-or-none eating pattern in reference to individual meals offered during this study.

When consumption was related to rehydratability or fat content, the slope of the straight line of "best" fit differed from a hypothesized zero slope in each case. The usefulness of this observation is questioned since experience tells us to expect a quadratic relationship between the factors related in these examples. To study the relationship between consumption and such factors as rehydratability or fat content, it would be more valid to vary these factors over a given range in one food item (holding all other variables constant) while measuring the consumption of that food item. Such a procedure, however, would not have practical meaning, since by so doing the "normal" meal pattern and eating environment would be significantly altered.

None of the measurements examined in this study proved useful for predicting consumption of foods by

the subjects. The acceptability rating technic was originally developed for predicting food preferences in large groups under conditions very different from those of this study. The validity of using this technic for predicting consumption in small groups under special conditions is open to doubt. Statistical criticisms offered in this paper reveal some of the limitations of applying this technic under these conditions. This technic has been used in previously reported studies of this type, primarily because no more adequate method was available. There is, therefore, a need to develop a technic which will accurately predict consumption of foods developed for small, specialized groups such as aerospace pilots in operational situations.

It has been suggested⁵ that aerospace pilots might be interviewed by experienced food psychologists to define their food habits and preferences. A separate taste panel, consisting of persons matching each individual's food traits, could then be formed and be available in the food laboratory for testing during all stages of the development of his food. This panel would augment the technical taste panel. Regardless of the screening method used during the development of these special purpose foods, ultimately, all foods should be evaluated under simulated operational conditions by those individuals who will be expected to consume them. Care should be taken, however, not to fatigue the acceptance of these foods by over-prolonged studies. It is highly unlikely that any absolutely foolproof method for predicting food consumption under actual aerospace operational conditions will be developed in the near future. Therefore, emphasis should be placed on development of additional varieties of foods and design of feeding systems which provide alternative foods and menus for selection by pilots during flight. Such development becomes increasingly critical with increasing flight duration.

CONCLUSIONS

A feeding system composed primarily of 21 bite-size foods supplemented with fruit juices is feasible for use as a survival-type ration in conditions simulating spacecraft operations. Because an aerospace feeding system based on bite-sized foods entails a minimum of operational problems, it would be worthwhile to develop such a system by increasing the variety and acceptance of bite-size foods.

A feeding system can be adapted to an individual's nutritional requirements by adjusting the portion size of one macronutrient-balanced food item.

There is a need to develop a technic to reliably predict consumption of foods developed for small, specialized groups, such as aerospace pilots in operational situations.

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Study of Man During a 56-day Exposure to an Oxygen-Helium Atmosphere at 258 mm. Hg Total Pressure

IX. Nutritional Evaluation of Feeding Bite-size Foods

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A feeding system composed of beverages and bite-size foods was used to support four flight crew members for 72 days. During the experiment, the crew spent 56 consecutive days in a 4-man space cabin simulator at 258 mm. Hg total pressure, including 175.2 mm. Hg P_{O_2} and 73.9 mm. Hg P_{He} . Energy was supplied to each crew member proportionally to his lean body weight. Metabolic balance studies were performed every 4 days of the experiment. The digestibility of energy and protein was low and was probably caused by the high melting point of the fat used in the formulation and coating of the bite-size foods. Fecal fat levels were high, however, there were no indications that a gastrointestinal disorder was associated with the low fat absorption. These studies indicate that bite-size foods are useful in supporting men in simulated aerospace conditions, but, new coatings are required to allow maximum digestibility of nutrients with minimum waste production.

FOODS DEVELOPED for aerospace feeding systems must provide the pilot with life support and satiety, be easily digested and assimilated, and promote a minimum production of fecal matter and flatus. During short-term space flights, 14 days or less, a diet containing a normal balance of carbohydrates, fats, proteins, and H_2O is sufficient for adequate life support. For long-term space flights, all nutrient requirements must be met by the diet to insure that deficiencies do not impair the pilot's mental and physical performance. Of equal importance is the satiety factor, since the pilot must willingly eat the food to obtain the nutrients and the psychological benefits of food are well known.

The weightlessness environment and limited capacity of space vehicles interfere severely with personal hygiene. Defecations while wearing a pressure suit have reportedly required an hour to accomplish and stowing the waste material becomes cumbersome and requires vital storage capacity. Problems associated with flatus production are intensified by reduced at-

mospheric pressure and the sealed environment. Trapped intestinal gas can cause incapacitation of the pilot as a result of distension, and the resulting flatus contaminates the atmosphere. It is critical, therefore, that proposed aerospace feeding systems be thoroughly evaluated to determine their complete usefulness. This paper describes the nutritional evaluation of a bite-size feeding system developed for use in space vehicles. The research was performed in conjunction with a 56-day atmosphere validation study which evaluated the use of an $O_2:He$ atmosphere. A further description of the overall study is found in reference 8.

EXPERIMENTAL PROCEDURE

A feeding system composed of 21 bite-size foods, 5 fruit juices and 3 combinations of the juices, an enriched drink, coffee, tea, sugar, and a vitamin-mineral tablet was used to support 4 flight crew members for 72 days. As part of the experimental procedure, the crew members spent 4 days on the diet while training, and 68 consecutive days in a 4-man space cabin simulator, of which 56 consecutive days consisted of exposure to 258 mm. Hg total pressure, including 175.2 mm. Hg P_{O_2} and 73.9 mm. Hg P_{He} . An attempt was made to provide each crewman with his individual caloric requirements. Each crewman's caloric needs were estimated by providing 45 kilocalories per kilogram of lean body weight with an additional allowance of 600 kilocalories for exercise. Since caloric values for the foods were not available prior to the study, estimates were obtained from the literature. Adjustments in caloric levels in the diet were made by varying the quantity of enriched drink. Each crew member was advised to consume as much of his diet as was possible, but no requirements were made concerning total consumption of all food. Data acceptability and consumption are reported in a separate manuscript, together with information concerning the food development.⁹

Each portion of the bite-size foods was weighed to the nearest hundredth of a gram on a top-loading electric balance and placed in a polyethylene bag. Beverages were also weighed to the nearest hundredth of a gram and placed in 480 ml. Mason jars. All food was identified with the crewman's name and date and

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supplied to the crewman daily. Food which was not consumed on the designated day was returned and again weighed to the nearest hundredth of a gram.

TABLE I. CHEMICAL ANALYSIS OF FOODS

Food item	% Nitrogen	% Fat	Kcal./gm.
Bacon square	10.40	26.64	5.75
Chicken sandwich	3.83	50.47	6.99
Beef sandwich	3.97	65.16	7.55
Cheese sandwich	4.06	45.96	6.82
Chicken bite	8.06	40.66	6.72
Beef bite	7.74	42.05	6.84
Bacon and egg bite	5.66	44.43	6.74
Gingerbread	1.30	22.59	5.11
Pineapple fruit cake	1.43	22.28	5.21
Date fruit cake	1.70	23.45	5.13
Brownie	2.68	18.64	6.02
Peanut cube	3.42	32.10	5.94
Apricot cube	1.83	30.96	5.52
Pineapple cube	2.69	26.11	5.22
Strawberry cube	1.43	16.28	4.92
Apricot cereal cube	1.79	16.08	4.94
Potato chip cube	1.43	24.57	5.33
Chocolate ice cream	1.56	38.06	5.85
Cinnamon toast	1.81	40.92	6.25
Toast	2.80	18.58	5.20
Candy	2.48	23.81	5.36
Enriched drink	1.84	12.95	4.62
Orange juice	1.01	1.09	3.73
Pineapple juice	0.41	1.47	3.76
Grapefruit juice	0.79	1.89	3.59
Apple juice	0.04	2.29	3.75
Grape juice	0.31	2.02	3.69

TABLE II. NUTRIENT INTAKE

Subject	66	67	68	69
Grams/day				
Protein	91.0 (16.0)*	97.5 (15.1)	105.9 (15.3)	87.7 (15.0)
Fat	117.2 (20.5)	106.7 (16.6)	142.7 (20.6)	114.9 (19.6)
Carbohydrates	356.0 (62.5)	434.1 (67.3)	437.5 (63.2)	378.2 (64.5)
Apparent digestibility of nutrients				
Per cent				
Protein	90.0	88.1	85.4	89.0
Fat	83.6	77.5	78.4	85.5
Carbohydrates	95.8	95.9	95.1	96.0

*Values in parentheses indicate per cent of diet.

TABLE III. WEIGHT AND BODY COMPOSITION CHANGES

Subject	66	67	68	69
Kilograms				
Body weight 4 Oct 1965	69.46	64.38	80.57	75.85
Body weight 9 Dec 1965	65.76	64.25	79.64	73.57
Weight change	-3.70	-0.13	-0.93	-2.28
Lean body weight 4 Oct 1965	56.28	53.85	63.34	60.42
Lean body weight 9 Dec 1965	55.64	53.15	63.79	59.59
Lean body weight change	-0.64	-0.70	+0.45	-0.83

Samples of all foods and beverages were taken each week for analytical analysis. These samples were frozen with dry ice and ground in a frozen state with a laboratory mill. This procedure was necessary to eliminate problems in grinding the high fat foods. All fecal specimens were collected in a waterproof cardboard container and saved for analysis. Fecal samples were weighed to the nearest hundredth of a gram and exactly one-fourth of each sample was combined into a 4-day composite which was dried in a forced air oven at 57°C. until weight changes on consecutive weighings were less than 1 per cent. The samples were then frozen with dry ice and ground with a laboratory mill. All urine voided was collected, stored at 4°C., and, later, a 4-day pooled sample containing no preservative was frozen and saved for analysis.

Metabolic balance studies were accomplished every 4 days of the experiment for energy, protein, calcium, phosphorus, magnesium, sodium, and potassium. Data on the electrolytes are being reported with the data on renal investigations.⁷ Energy determinations were accomplished on food, feces, and urine samples using a Parr adiabatic bomb calorimeter. The urine samples were prepared for combustion in the calorimeter by freeze-drying. Nitrogen determinations were accomplished on food and fecal samples by the Dumas method with the use of a Coleman Model 29A nitrogen analyzer. Urinary nitrogen was determined with an "F&M" carbon, hydrogen, nitrogen analyzer Model 180. Fat determinations on foods and feces were accomplished by chloroform-methanol extraction.⁶

The crew members were weighed and body composition measurements were performed at weekly intervals prior to entering the simulator and again after leaving the simulator.¹

RESULTS

Analytical data obtained from the analysis of each of the food items used in the feeding system are reported in Table I. Many of the food items contain high levels of fat which increased the caloric content above that originally anticipated. An average of 3,655 gross calories were offered to each crewman per day which was approximately 400 calories more than original estimates obtained from the literature. Average daily nutrient intakes for protein, fat, and carbohydrates are shown in Table II. These values show a normal distribution of fats, carbohydrates, and protein for each individual. All subjects tended to select foods which were low in fat and thus fat intake levels were lower than anticipated.

Data on the apparent digestibility of nutrients are also shown in Table II. These data show an unusually low digestibility for fat. The digestibility for protein is also low and only the carbohydrate digestibility is normal considering the low fiber content of the food items.

Weight and body composition changes are recorded in Table III. Two crewmen, 66 and 69, lost considerable body weight while losses for the other two were less severe. Measurement of body composition indicates that these losses were primarily fat. Data on

energy utilization are shown in Table IV. These data illustrate a poor digestibility for energy which is caused primarily by the low utilization of the dietary fat. Energy requirements for each individual were calculated using data from body composition changes. Considering the fact that Subject 66 expended approximately 100 kilocalories¹¹ more than the other crew members each day for exercise, a reasonable correlation exists between lean body weight and caloric requirements. The final line of Table IV shows that estimates of caloric requirements made before the initiation of the study were too high.

Nitrogen balance data are given in Table V. Nitrogen losses in the sweat, hair, fingernails, and skin were not directly measured and therefore, all the crew members had an apparently high positive nitrogen balance not indicative of growth in lean body weight.

Observations made on the effects of the diet on the gastrointestinal tract of the crew members indicated the diet had a low compatibility for space use. The data reported in Table VI show that each subject had an average of 1 fecal specimen for each day on the diet. The majority of specimens were nonformed, had a pungent odor, and reportedly caused difficulty with personal hygiene. Note the unusually low water content of the fecal matter considering that most stools were nonformed. The fat level in the specimens was unusually high for a diet of a moderate fat intake. Clinical tests for indications of malabsorption of fat, such as urinary indicans, were normal. During the low pressure phase of the experiment, the crew members reported problems with flatus production. Upon several occasions, distention caused by gas in the gastrointestinal tract became so severe that the crew members could not perform in an efficient manner.

DISCUSSION

A feeding system composed of beverages and bite-size foods proved to be useful in providing life support for crewmen in a simulated space environment. Metabolic balance studies revealed that the food was utilized at less than normal levels and created gastrointestinal effects which were incompatible with efficient performance in an orbiting vehicle. The digestibility of energy averaged about 88 per cent which is low for a low residue diet.^{2,10} Since weight and volume are critical in space systems, this diet would not be judged optimum for space use.

The poor utilization of energy was due to the low digestibility of fat. The fat used in the formulation and as a coating for many of the bite-size foods was obtained by fractionation of palm kernel oil and had a melting point range between 136° and 141°F., much above body temperature. Analysis of the fatty acids obtained from the saponification of the fat coating and fecal samples, using a gas chromatograph, showed good agreement for length of carbon chain and degree of saturation in both food and fecal fat. Crockett and Deuel⁵ have reported that the digestibility of fat decreases rapidly as the melting point exceeds 124°F. These investigators also suggested that the formation

of insoluble soaps increases with length of carbon chain. Since soaps are not removed using an ordinary solvent extraction procedure, it is possible that additional lipid material was present in the fecal samples and thus, the digestibility of fat may have been even lower than apparent.

In view of the slight losses in lean body weight, the large positive nitrogen values can probably be explained by losses in sweat, keratinized tissue, et cetera, which were not directly measured. Considering the exercise level of the crew members, these losses are in agreement with data reported by others.^{3,4} These data show a need for direct measurement of losses of nitrogen in sweat and keratinized tissue in future simulator studies.

The frequency of defecation and the extent of flatus production associated with the use of this feeding system showed that it lacked compatibility for manned orbital space flight. It is particularly desirable to reduce the frequency of defecation to fewer than one

TABLE IV. ENERGY UTILIZATION

Subject	66	67	68	69
Kilocalories/day				
Gross caloric intake	2937	3269	3521	2987
Caloric loss feces	309	378	485	284
Digestible energy	2628	2891	3036	2703
Coefficient of digestibility	89.48	88.45	86.23	90.49
Caloric loss urine	11	11	11	10
Metabolizable energy	2617	2880	3026	2693
Energy balance*	-456	+35	-93	-248
Metabolizable energy requirements	3073	2845	3119	2941
Estimated energy requirements†	3132	3149	3450	3319

*Calculated from body composition changes using 9 kcal./gm. of fat weight changes and 4 kcal./gm. for lean weight changes.

†45 kcal./kg. of lean body weight +600 calories for exercise.

TABLE V. NITROGEN BALANCE

Subject	66	67	68	69
Grams/day				
Intake	14.56	15.60	16.94	14.03
Fecal loss	1.45	1.85	2.47	1.55
Urinary loss	9.78	10.18	10.14	9.10
Balance	+3.33	+3.57	+4.33	+3.38

TABLE VI. FECAL DATA

Subject	66	67	68	69
Number of specimens for 72-day period	73	72	72	79
Water content in per cent	34.2	37.3	36.3	34.5
Dry matter grams/day	43.3	53.5	67.1	41.5
Per cent fat	44.3	44.8	45.5	40.0
Per cent nitrogen	3.35	3.46	3.68	3.73
Kilocalories/gram	7.14	7.07	7.22	6.84

every 2 days. The excessive production of flatus was here traced to certain beverages.

SUMMARY

A feeding system composed of beverages and bite-size foods is capable of providing sustenance for long-term space missions. The fat coatings presently used to maintain the integrity of bite-size foods are poorly utilized and may be the factor responsible for frequency of defecations. The development of new coatings for bite-size foods is necessary before an all bite-size feeding system can be recommended for manned space flights.

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Study of Man During a 56-day Exposure to an Oxygen-Helium Atmosphere at 258 mm. Hg Total Pressure

X. Enteric Microbial Flora

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One hundred twenty-four fecal samples collected during various phases of the study were examined to determine any changes which may have occurred in the bacterial flora. The counts of all microorganisms remained within the normal range except those for the enterococci. There was a decrease in the number of enterococci as the subjects went on the experimental diet, but the values returned to normal as the regular diet was resumed after the 56-day flight. The change observed was not considered to be of clinical significance.

AS PART OF AN EXTENSIVE study of the effects of long term exposure to an oxygen-helium atmosphere on man, we were able to monitor the microbial flora of the fecal material of the experimental subjects. The objectives were to determine if any changes in the flora might occur as a result of the prototype diet used or the environmental conditions in the space cabin simulator, and to evaluate any alterations observed in terms of their possible clinical significance.

MATERIALS AND METHODS

Six healthy young adult male subjects participated in the study. Four of these men were confined for 56 days in an atmosphere of 70 per cent oxygen and 30 per cent helium at a total pressure of 258 mm. Hg. The other 2 subjects served as ground controls, i.e., remained at ambient conditions but consumed the same diet as those in the chamber. The nutritionally adequate diet consisted of compressed freeze-dried cubes

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of various foods. Detailed information concerning the diet and the environmental conditions will be published elsewhere.

To examine the effects of these factors the study period was divided as shown in Table I. Baseline values were obtained during phase 1, and the effect of the diet was determined in the next period. Phase 3 incorporated the additional factor of the test atmosphere. Ideally it would have been desirable to have samples while the men were on the diet at ground level after the chamber exposure, but the experimental protocol did not permit this.

The samples were analyzed within 3 hours after defecation. A 10 gm. portion of feces was homogenized in 90 ml. of fluid thioglycollate with a Waring blender. The thioglycollate medium was used for this and all subsequent dilutions (10^{-2} to 10^{-10}) to protect the anaerobic bacteria from oxygen damage. Appropriate dilutions of the sample were then inoculated in duplicate in the culture media shown in Table II. Pour plates were made with the media for all the anaerobic bacteria except the lactobacilli. The incubation time and temperature varied according to the medium used and the microorganisms to be isolated. Pagano Levin plates were incubated at 25°C. for 5 days, while media for aerobic bacteria were incubated at 36°C. for 24 to 48 hours. Incubation of cultures for anaerobic bacteria was continued for 7 days at 36°C. in an atmosphere of 10 per cent carbon dioxide and 90 per cent nitrogen gas. Final identification of isolates was based upon colonial and cellular morphology, tinctorial properties and cultural characteristics.

RESULTS AND DISCUSSION

The median values and range of bacterial counts (given as logs) for the major groups of bacteria recovered from the subjects are shown in Figure 1.

Fig. 1. Log of median values and range of bacterial counts.

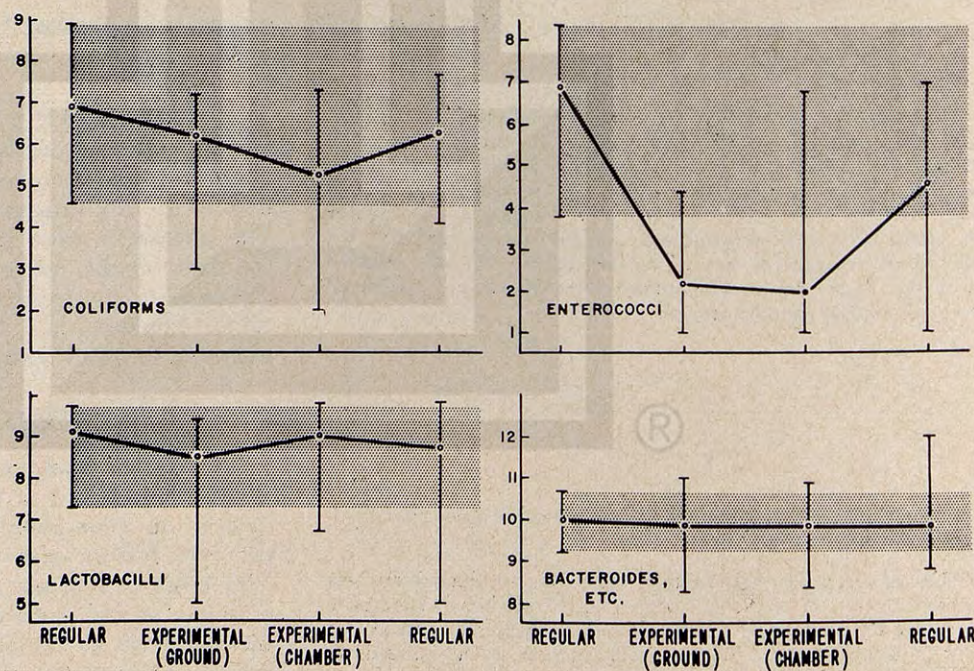


TABLE I. OUTLINE OF THE STUDY

Phase	Diet	Environment	Number of Samples	Days' Duration
1	Regular	Ground	30	14
2	Experimental	Ground	30	12-21*
3	Experimental	Chamber	49	56
4	Regular	Ground	15	7

*Two ground controls—21 days.

TABLE II. MEDIA USED FOR CULTURING THE VARIOUS MICROORGANISMS

Media	Microorganisms
For aerobic bacteria	
Levine EMB agar	Coliforms, Proteus,
MacConkey agar	Salmonellae, etc.
Mannitol Salt agar	Staphylococci
KF Streptococcus agar	Enterococci
Mitis Salivarius agar	Streptococcus salivarius
Pagano Levin agar	Yeasts, etc.
Blood agar	Diphtheroids
(Trypticase Soy agar + 5% human blood)	
For anaerobic bacteria	
Fluid thioglycollate + 1.5% agar	Bacteroides, etc.
SPS agar	Clostridia
Blood agar	Clostridia
Rogosa SL agar	Lactobacilli

These are the organisms which are normally present in greatest number. The shaded areas represent the normal range of counts actually found in the samples from the subjects in this study. The values agree with those reported by other workers.¹⁻⁴ The results show a decrease in the enterococci below the normal minimum as the subjects went on the experimental diet, but the counts returned to normal as the regular diet was resumed. This shift was found in each of the test subjects. The additional factor of the chamber environ-

ment produced no observable effect on the flora. No change in the occurrence of any other microorganisms was observed, including not only those plotted here but all others encountered, i.e., staphylococci, clostridia, yeasts, etc. Staphylococci were isolated from 32 of the 124 samples examined; counts per gram ranged from less than 100 to approximately 10,000. *Streptococcus salivarius* was isolated from about 90 per cent of the samples. Yeasts recovered included *Candida*, *Saccharomyces* and *Torula* species. No special attempts were made to enumerate the diphtheroids, though they were observed occasionally on blood agar plates incubated aerobically and anaerobically. The counts of clostridia ranged from 3.0 to 9.0 (logs) with a median value of 4.0. This figure is within the range reported by others.^{2,3,4} Changes in the fecal flora may be associated with physiologic stress, diet, infectious disease, antibiotic or drug therapy. Such alterations could result in the suppression of harmless bacteria and facilitate the growth of pathogens; a phenomenon especially important in carriers of viral and bacterial pathogens. There was no evidence at any time of the emergence or presence of enteric pathogens such as *Salmonella* or *Shigella* species. It is emphasized that isolation procedures used were sufficiently sensitive to detect those pathogens, even had they been present in very low concentration.

Other investigators have suggested that changes in the diet are accompanied by, or may even cause, changes in the fecal flora. In this connection, Gall and Riely¹ reported a shift in the predominating anaerobic flora which they attributed to changes in diet

rather than environmental factors. Should a causal relationship exist, it may follow that if the diet remains constant so will the flora. It was interesting to note that while the dietary components remained constant for each individual during the experimental phase, normal fluctuations were still evident in the fecal flora. In earlier unpublished studies we have examined the fecal flora of subjects on a variety of formula diets and in each case no significant qualitative or quantitative changes in the flora were observed.

We are aware of no report in the literature in which clinical significance has been assigned to alterations of this magnitude. The changes reported here in the enterococci are probably not statistically significant in view of the extreme normal fluctuations, but, in any event, we have no reason to attach any clinical importance to these findings. Based on the results of this study, we anticipate no problems arising from changes in the fecal flora due to this diet or the environmental conditions employed.

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Study of Man During a 56-day Exposure to an Oxygen-Helium Atmosphere at 258 mm. Hg Total Pressure

XI. Oral, Cutaneous, and Aerosol Bacteriologic Evaluation

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Studies were initiated to determine the numbers, distribution, and types of microorganisms encountered under conditions of a sealed environment for extended periods of time, such as would occur during space explorations. A 56-day experiment, utilizing four test subjects confined within a double-walled test cell in an oxygen-helium atmosphere at 258 mm. Hg, was performed. Quantitative counts of the aerobic microorganisms present in the circulating atmosphere as well as those present on the skin of the subjects were established. Distribution of coagulase positive, phage typable *Staphylococcus aureus* strains and predominant microbial types in throat, nasal, skin and aerosol samples was determined. Evidence of a staphylococcal transfer between subjects was obtained. Implications of these findings, as related to the utilization of the two-gas atmosphere for future space flights, are discussed.

THE STUDY of microbiological problem areas that might be of concern to the health and well-being of astronauts during long-term habitation of outer space, either in orbiting or land-based stations, is essential. Many of the anticipated problems are amenable to examination through the use of environmental test cells specifically designed and constructed for prolonged human occupancy. The considerations for utilizing a two-gas atmosphere for extended manned space flight, with helium as the inert diluent, have been reviewed by Welch and Robertson,⁵ and Clamann.³ The microbiological investigations described were undertaken to determine the effects of a 56-day exposure to an oxygen-helium atmosphere at 258 mm. Hg total pressure⁴ on the oral and cutaneous microbial populations of man. Additionally, aerosol studies were performed to determine the numbers and types of microorganisms present in the circulating atmosphere.

METHOD

Briefly, the experiment was conducted on four test

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subjects confined within the double-walled environmental chamber in operation at the USAF School of Aerospace Medicine, Brooks AFB, Texas. Experimental parameters, along with a description of the test cell and subjects, are detailed elsewhere.⁴

Nasal, throat, and skin samples were collected from each individual prior to, during, and following confinement in the test cell and subjected to bacteriological analyses to determine any significant alterations in the aerobic microflora. Subjects collected all specimens during the test period. During the interval of confinement nasal, throat, and skin samples were collected twice weekly on Monday and Thursday. During the pre-experimental and post-experimental periods, specimens were taken more frequently to establish adequate baseline data.

a. Throat swabs, immersed in trypticase soy broth as a preservative, were streaked to 5 per cent sheep blood agar plates, incubated at 35°C. for 24 hours, and observed for gross changes in the total flora.

b. Nasal swabs, also immersed in trypticase soy broth, were streaked to sheep blood agar as well as mannitol salt agar to detect the presence of *Staphylococcus aureus* organisms. Suspicious staphylococci (at least 3 colonies) were picked to trypticase soy agar (TSA), and retained for bacteriophage typing.

c. Skin specimens were collected from the medial aspect of the upper arm. These samples were obtained by means of a specially designed contact plate (Rodac, Falcon Plastics) containing 5 per cent sheep blood agar in which the surface of the agar is pressed directly against the skin area to be studied. Subjects alternated arms so as to minimize errors in the sampling technic. All plates were incubated aerobically for 48 hours at 35°C. before counts were performed.

Five aerosol sampling stations were established within the test cell. Blood agar petri plates were opened by the test subjects and exposed to the cabin atmosphere for periods of one hour. An unexposed blood agar plate was passed into the chamber to serve as a control for changes in atmospheric pressure. The plates were removed from the test cell and incubated aerobically at 35°C. until colony counts could be made. Predominant microbial species were determined and hemolytic *Staph. aureus* colonies (3 from

each plate) were streaked to TSA and retained for phage typing.

The retained staphylococcal colonies were restreaked for purity on a blood agar plate, and single colonies were tested for coagulase reaction by the tube method. All coagulase positive staphylococci were phage-typed by the procedure of Blair and Williams.² The phages employed were the international basic set: 29, 52, 52A, 79, 80, 3A, 3B, 3C, 55, 71, 6, 7, 42E, 47, 53, 54, 75, 77, 83A, 42D, 187, and 81. The experimental phage UC-18¹ was also used. All phages and propagating strains were obtained from the Communicable Disease Center, Atlanta, Georgia. The routine test dilution of each phage was employed; that is, the highest dilution of phage lysate which just failed to give confluent lysis. Only reactions of 2+ (more than 50 plaques) were considered in reporting a phage pattern.

RESULTS

Throat Cultures. No shift in the normal distribution of microorganisms was noted. The subjects harbored the usual predominant flora consisting of alpha streptococci, neisseria, and *Staphylococcus epidermidis* species. Occasionally, *Staph. aureus* colonies were observed in small numbers. Though specifically looked for, beta hemolytic streptococci were not isolated.

Nasal Cultures. Table I shows the staphylococcal

TABLE I. STAPHYLOCOCCAL PHAGE TYPES FROM NASAL CULTURES

Day of Experiment	66	69	67	68
-28	29/52/80	7/53	*	None
-27	29/52/80/81	7/53	29	None
-26	29/52/80/81	7/53	29	None
-23	29/52/80/81	*	None	None
-22	29/52/80/81	7/53	29	None
-20	*	7/53	29	*
-16	29/52/80/81	7/52A	None	None
-13	29/52/80/81	7	29	None
-9	29/52/80/81	7;7/52A	None	None
-6	29/52/80/81	7/52A	None	None
-5	29/52/80/81	7	None	None
1	29/52/80/81	7	29	None
2	29/52/80/81	7;7/53	None	None
5	29/52/80/81	7/53	None	None
8	29/52/80/81	7;7/53	29	None
12	29/52/80/81	7/53	29;7	None
15	29/52/80/81	7/53;29	29	29
19	29/52/80/81	7/53	29	29
22	29/52/80/81	7/53	29	29
26	29/52/80/81	7/53	29	29
29	29/52/80/81	7/53	29	29
33	29/52/80/81	7;7/53	29	29
36	29/52/80/81	7	None	29
40	29/52/80/81	7;7/53	None	None
43	29/52/80/81	7	None	None
47	29/52/80/81	7/47	29	29
49	29/52/80/81	None	None	29
54	29/52/80/81	7/53;29	29	29
+1	29/52/80/81	29	29	29
+2	29/52/80/81	7/53;29	29	29
+3	29/52/80/81	7/53;29	29	29
+5	29/52/80	7	29	29
+6	*	*	None	29
+7	*	*	None	None
+8	29/52/80	7/53	None	None

*No specimen received

phage types isolated from each of the four test subjects during the course of the experiment. The minus and plus signs in the left hand column (Day of Experiment) refer to pre- and post-experimental periods when the subjects were at ground level under normal atmospheric conditions. Three of the subjects harbored specific, identifiable staphylococcal strains, while the fourth subject (Number 68) apparently was not a carrier of any *Staph. aureus* strains. Both blood agar and mannitol salt agar plates from nasal cultures of this individual were negative for this organism. To assure that no *Staph. aureus* organisms were present, colonies were streaked to TSA, tested for coagulase reaction, and phage typed. All cultures proved to be coagulase negative, nontypable *Staph. epidermidis* strains.

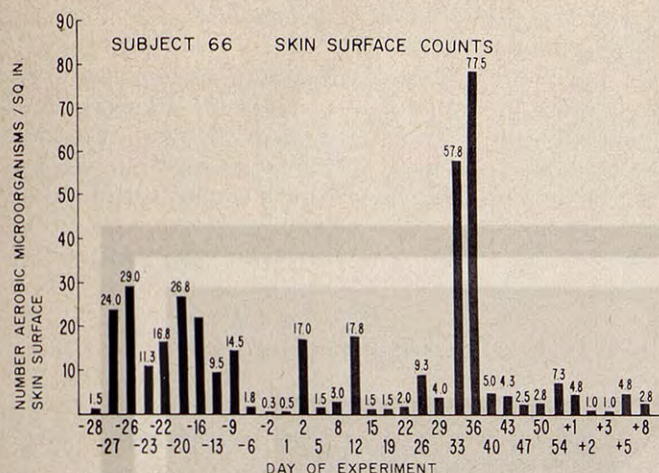
Following two weeks confinement at the simulated altitude, a transfer of staphylococcal phage type 29 from subject 67 to subject 68 was evident. Nasal cultures streaked to mannitol salt agar revealed large numbers of these microorganisms. The transfer persisted throughout the course of confinement and into the post-experimental period. It is of interest to mention that subjects 67 and 68 were on the same sleep/rest/work cycle; hence, were closely associated with one another.

Lesser evidence for a transfer of phage type 29 from subject 67 to 69 is present. Although this phage type was isolated from the nasal cultures of subject 69 on five occasions (15th, 54th, +1, +2, and +3 days), the transfer would appear to be of a transitory nature. Not enough post-experimental cultures were obtained, however. Subject 66 retained his phage pattern throughout the experiment.

Staphylococcal Phage Types from Aerosols. Table II presents the staphylococcal phage types isolated from blood agar plates exposed to the test cell atmosphere for periods of one hour while all four subjects were awake. It is interesting to note that all phage patterns harbored by the subjects were isolated from aerosol samples at least once during the period of confinement. The more frequent isolation of phage

TABLE II. STAPHYLOCOCCAL PHAGE TYPES FROM AEROSOLS

Day of Expt.	Phage Type(s)
-7	None
-5	7
5	29/52
7	None
12	None
14	29
16	29;29/52
26	29;29/52/80/81
28	29;7/53
30	29
33	29;7/29
35	None
37	None
47	29
49	7/53
51	29
54	29
56	29
+2	None



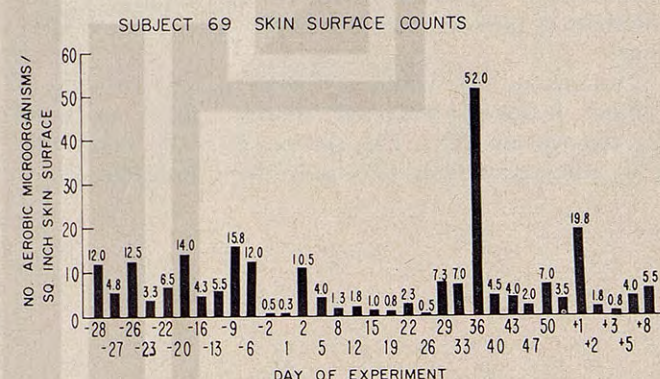
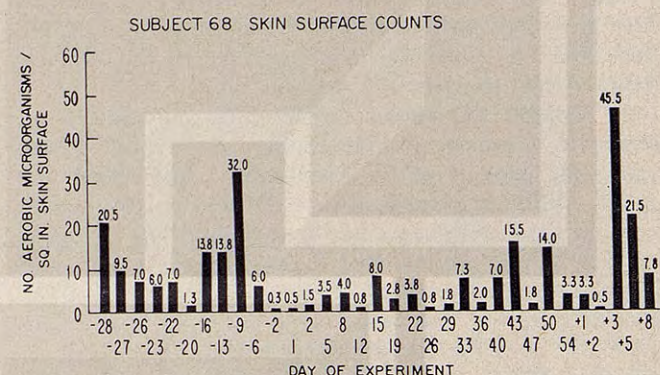
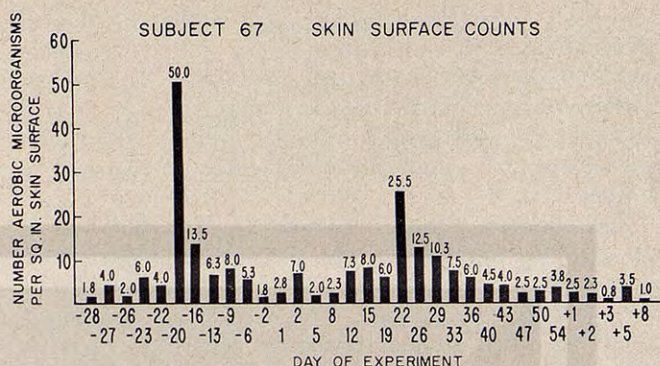
type 29 (10 of 19 sampling days) may account for its greater transmissibility among subjects.

Skin Samples. Figures 1 through 4 represent data obtained on the numbers of aerobic microorganisms isolated per square inch skin surface of the area tested. The medial aspect of the upper arm, an area relatively free of hair, was chosen as the skin sampling site, since previous studies of other areas had shown that the number of microorganisms present was directly related to the hirsuteness of the individual. During the period of confinement the subjects took one sponge bath per day prior to their sleep cycle. *Staph. epidermidis* was always greatly in the predominance of the organisms isolated from the skin cultures. Other organisms found in small numbers included *Staph. aureus*, an unidentified gram positive rod, probably a diphtheroid, and various saprophytic fungi.

Aerosol Counts. The total number of aerobic microorganisms present per square inch cabin surface area per hour may be found in Figure 5. Air flow through the cabin during the sampling period was approximately 250 std. cu. ft. per min.; however, some variation was present depending upon the number of blowers in operation. The individual counts shown in Figure 5 were obtained by averaging the total number of microorganisms found at the five aerosol sampling stations and dividing by the area of the blood agar plate. Data for the 40th, 42nd, and 44th days are not included, since the blood used to prepare the agar plates that week was contaminated. While the predominant organism isolated from aerosol samples was *Staph. epidermidis*, many more *Staph. aureus* colonies were noted than in the skin counts. In several instances *Staph. aureus* colonies comprised one half or more of the total colonies counted. Various *Bacillus* sp. and saprophytic fungi were found in lesser numbers.

DISCUSSION

Immediate postexperimental radiographic studies of subject 68 revealed changes in the left frontal and left maxillary sinuses, and the subject was placed on a two-week regimen of erythromycin therapy. Throughout the entire experimental period, however, the subject was asymptomatic, with no clinical signs of an acute



Figs. 1 through 4. Aerobic skin microorganisms from fifty-six day experiment in an oxygen-helium atmosphere at 258 mm. Hg total pressure.

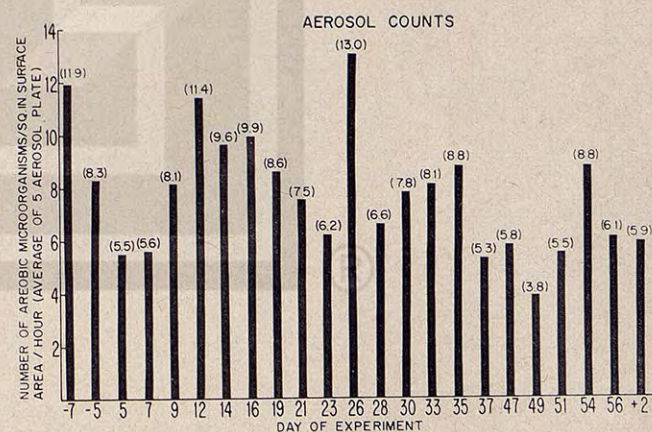


Fig. 5. Aerobic microorganisms isolated from circulating atmosphere during fifty-six day experiment in an oxygen-helium gas mixture at 258 mm. Hg total pressure.

sinusitis.⁶ Subsequent radiographic studies on this individual since his return to his duty station have been negative; however, nasal cultures reveal that he still harbors phage type 29. These findings appear to indicate that the radiographic changes initially noted were not related to the presence of the phage typable staphylococci.

The cutaneous studies suggest that no build-up of the aerobic microflora of the skin occurred during the course of the experiment. The counts are quite uniform with few exceptions. The occasional high counts found for each subject probably occurred when for some reason the subjects were unable to wash adequately. The unusually low counts found on minus 2 days are adequately explained by an entry in one subject's diary in which he mentions that all the subjects received two showers the previous day.

As regards the aerosol studies, no cumulative increase or decrease in the numbers of aerobic microorganisms present in the circulating atmosphere was noted. While it is realized that the open plate method of sampling atmospheres has limitations, plates exposed by this technic consistently showed higher counts and more representative types of microorganisms than those obtained during the same time interval by a commercially available impinger-type sampler placed externally. Moreover, it is believed that the open plate method is of considerable value in determining relative numbers of microorganisms over an extended period of time.

Portions of the cabin air were passed through a lithium hydroxide-activated charcoal filled cannister for removal of CO₂. This device, fitted on both ends with a fiberglass filter (Aerosolve 95, Cambridge Filter

Corp.), no doubt acted as a microbiological filter, reducing the total count.

From a microbiological viewpoint there appears to be no serious objections to the use of an oxygen-helium atmosphere at 258 mm. Hg total pressure. The one demonstrated instance of staphylococcal interchange was not occasioned by any adverse clinical symptoms of consequence. For aesthetic purposes and odor problems, a sponge bath daily is considered sufficient to keep the resident skin microorganisms in check.

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Study of Man During a 56-day Exposure to an Oxygen-Helium Atmosphere at 258 mm. Hg Total Pressure

XII. Clinical Observations

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The present report describes the clinical findings in four aircrew members who lived for 56 days in an oxygen-helium environment at 258 mm. Hg total pressure. During pre- and post-experimental periods, the subjects underwent a complete medical evaluation to include appropriate roentgenographic studies, electrocardiograms, electroencephalograms, and blood chemistries. During the 56-day test phase, clinical symptoms were minimal. Most bothersome to the subjects were increased amounts of flatus, only rarely causing abdominal discomfort from trapped intestinal gas. All of the individuals developed mucous membrane dryness associated with a decreased relative humidity in the chamber. Two subjects noted nasal congestion and none experienced middle ear problems. One individual developed mild elevations in transaminase enzymes which could not be explained. Resting electrocardiograms from another subject showed intermittent changes consistent with the Wolf-Parkinson-White syndrome, which could not be attributed to the environment. Daily exercise was performed on a bicycle ergometer without symptoms or signs of a decompression disturbance. At no time during the experiment were there any clinical disturbances which might have prevented the completion of a prolonged manned space mission.

METHODS

Four healthy male USAF aircrew members, ages 27 to 29 years, participated in the study. The physical characteristics of the subjects and the experimental profile have already been described.⁸ Prior to entering the chamber and immediately upon completing the postexperimental ground phase, the subjects underwent extensive physical examinations including ophthalmologic, ear-nose-throat, neurologic, and psychiatric evaluations. In addition, there were pre- and post-experimental chest, abdominal, and paranasal sinus roentgenographic studies; electrocardiograms; vectorcardiograms; and electroencephalograms. These studies were performed in the Clinical Sciences Division of the USAF School of Aerospace Medicine.

The subjects were taught to determine vital signs, perform venipunctures, and carry out 12 lead standard electrocardiograms on each other. Vital signs were recorded daily on each subject upon awakening. Venous blood, urine, and stool samples were passed to the outside through a small pass lock without altering the environmental conditions. A medical review of systems was performed twice daily in addition to the continuous monitoring of the experiment.

Numerous blood studies were performed at weekly intervals to evaluate the hematopoietic and hepatic systems and to complement those studies described in previous reports.^{3,7} Hemoglobin and hematocrit determinations, white blood cell and differential counts, cell morphology descriptions, reticulocyte counts, platelet counts, and red blood cell osmotic fragility curves were performed by standard methods. Fasting blood glucose, blood urea nitrogen, and alkaline phosphatase were determined by AutoAnalyzer techniques. Serum protein electrophoresis was performed with the Spinco Model R paper electrophoresis system and total serum proteins were measured by a modified Biuret method.¹³ Serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) were ascertained by the Sigma-Frankel procedure,¹² and serum bilirubin with fractionation by the method of Malloy and Evelyn.¹⁰ Bromsulphalein retention was determined pre- and postexperimentally utilizing standard dye injection techniques with a 45-minute venous blood measurement.

TO DATE THE SPACE cabin atmosphere utilized in United States manned space flight has consisted of a 100 per cent oxygen, 258 mm. Hg environment. With the prospect of more prolonged space ventures in the immediate future, the question of the addition of an inert gas to this somewhat hyperoxic environment still remains important. Before an environment can be used in the closed ecological system of a manned spacecraft, it must be suitable from a physiological and medical viewpoint. The present report will describe the clinical findings in four aircrew members who lived for 56 days in an oxygen-helium atmosphere at 258 mm. Hg total pressure. Appropriate comparisons will be made with clinical findings from previous hypobaric chamber studies.^{9,11}

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STUDY OF MAN IN OXYGEN-HELIUM ATMOSPHERE—XII. CLINICAL OBSERVATIONS—ZEFT, ET AL

TABLE I. CLINICAL FINDINGS DURING THE EXPERIMENTAL PHASE

Symptoms or Signs	Subject No.
Dryness of mucous membranes	66, 67, 68, 69
Nasal congestion	68, 69
Conjunctivitis	66, 68, 69
Increased flatus	66, 67, 68, 69
Abdominal pain from trapped intestinal gas	67, 68
Elevated transaminase enzymes	67
ECG pre-excitation syndrome	69
Paresthesias with exercise ¹⁶	67, 68, 69
Postexperimental sinus abnormality (x-ray)	68
Middle ear problems	0
Decompression disturbances	0

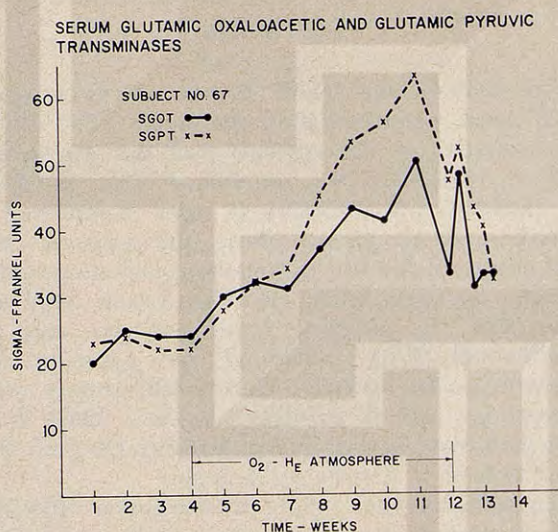


Fig. 1. SGOT and SGPT values for subject 67 plotted against time. Each data point represents a single laboratory determination. Values for both SGOT and SGPT greater than 40 units are considered abnormal.

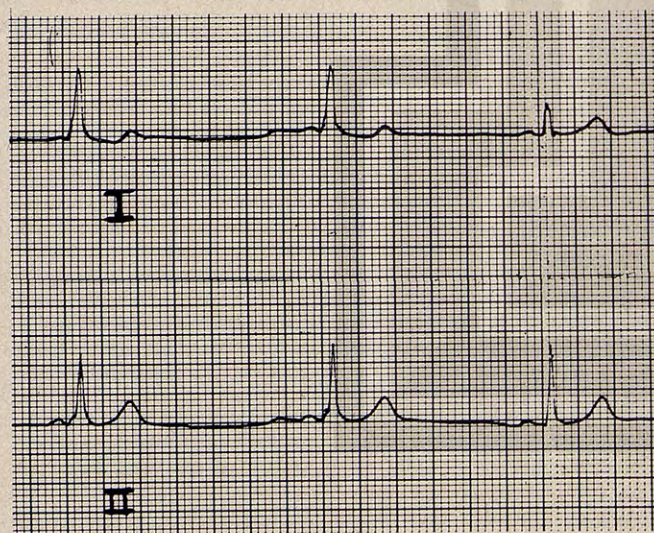


Fig. 2. Standard leads I and II of a resting electrocardiogram from subject 69 during the sixth experimental week. The first two complexes show the shortened PR interval, widened QRS, and delta wave of the pre-excitation pattern. The third complex is normal. Paper speed was 25 mm per second.

Freshly voided morning urine specimens were analyzed once weekly for sugar, protein, acetone, specific gravity, and pH. A microscopic examination was performed on the sediment. All stool samples were tested for occult blood with guaiac reagent.

RESULTS

Table I summarizes the clinical findings detected during the hypobaric oxygen-helium phase. All four individuals experienced some difficulty associated with dryness of mucous membranes. They described dry cracked lips often with minimal bleeding, a "dry velvety" feeling on touching the tongue to the hard palate, and a cooling sensation about the eyes frequently with signs of conjunctivitis. These symptoms were most noticeable when the relative humidity fell below 30 per cent. Relief was obtained with moistening agents such as Chap Stick® and methyl cellulose eye drops. Subjects 68 and 69 also had frequent symptoms of nasal congestion, occasionally with a mucous discharge containing small amounts of clotted blood. Subject 66 with known seborrhea had numerous exacerbations of this problem, specifically about the scalp and face.

Increased amounts of intestinal gas and flatus were bothersome to the subjects throughout the experimental phase. Although a slight increase in flatus was noted upon starting the experimental diet, the difficulty was greatly magnified during the low pressure, oxygen-helium conditions. During one afternoon, subjects 67 and 68 experienced rather severe, crampy mid and lower abdominal pain with inability to expel trapped gas. The pain was relieved after approximately 5 minutes in one subject and after 30 minutes in the other by their assuming a knee-chest position. The subjects implicated the supplementary fruit juices in their diet as the greatest offenders in precipitating these symptoms. Bacteriologic studies on fecal samples demonstrated decreased enterococci counts on the experimental diet but no change in fecal flora related to the hypobaric experimental period.⁶

During the oxygen-helium phase, transaminase enzymes from subject 67 revealed a small but progressive rise to a peak SGPT and SGOT of 63 and 50

TABLE II

Subject Number		Total Bilirubin (mg%)	Albumin/ Globulin	Alkaline Phosphatase (KA units)	SGOT (S-F units)	SGPT (S-F units)	BSP Retention (%)
66	Pre*	.6 ± .0	1.6 ± .1	7.3 ± .1	18 ± 0	14 ± 2	0
	Exp**	.6 ± .1	1.7 ± .1	7.0 ± .9	17 ± 1	13 ± 3	—
	Post*	.7 ± 0	1.7 ± 0	5.8 ± .3	16 ± 0	13 ± 1	1.8
67	Pre	.4 ± .1	1.8 ± .2	6.2 ± .4	24 ± 0	22 ± 0	0
	Exp	.7 ± .2	1.8 ± .1	6.5 ± .3	37 ± 7	45 ± 13	—
	Post	.9 ± .1	1.9 ± .1	5.7 ± .4	40 ± 9	48 ± 5	2.2
68	Pre	.9 ± 0	1.8 ± .1	6.0 ± .2	19 ± 2	12 ± 0	0
	Exp	1.1 ± .2	1.8 ± .2	5.7 ± .5	18 ± 4	13 ± 3	—
	Post	1.0 ± .1	1.8 ± .1	4.9 ± .3	18 ± 0	11 ± 3	3.8
69	Pre	.5 ± .2	2.2 ± 0	8.4 ± 1.0	26 ± 2	31 ± 0	3.0
	Exp	.6 ± .1	2.1 ± .2	9.2 ± 1.0	22 ± 2	26 ± 5	—
	Post	.8 ± .1	2.3 ± .3	9.4 ± .1	15 ± 1	16 ± 0	4.2

*Mean values and range for duplicate determinations; BSP retention is the result of a single test.

**Mean values and S. D. for 8 determinations.

Sigma-Frankel units, respectively, during the seventh experimental week (Figure 1). Subsequently, these enzyme values slowly declined to normal. No other abnormalities in liver function studies were detected (Table II). BSP retention pre- and postexperimentally was 0 and 2.2 per cent, respectively. Subject 67 was entirely asymptomatic and postexperimental examination immediately upon descent revealed no signs of hepatic tenderness or enlargement.

Resting electrocardiograms on subject 69 during the sixth experimental week showed an intermittent pattern of ventricular preexcitation or the Wolf-Parkinson-White syndrome (Figure 2). There was a shortened PR interval to 0.11 sec., a prolonged QRS to 0.13 sec., a characteristic delta wave, and a leftward shift of the mean QRS axis in the frontal plane on electrocardiograms. Previous and subsequent tracings failed to reveal this pattern. There was never a history of supraventricular tachycardia, and exercise electrocardiograms were not altered.

Hematologic studies were normal throughout the entire experiment (Table III). Red cell osmotic fragility studies, likewise, showed no deviation. Pre-experimentally, subject 67 had urethritis and asymptomatic pyuria which cleared after 10 days of sulfisoxazole therapy. Otherwise, urinalyses showed no deviation from normal. More detailed renal function studies have been described in an earlier report.⁷ Stool specimens were negative for occult blood throughout the study.

Postexperimentally subject 68 showed roentgenographic signs of mucous membrane thickening of the left frontal and maxillary sinuses. He never described symptoms of acute sinusitis, sinus outflow obstruction, or purulent drainage. After two weeks of erythromycin therapy, paranasal sinus x-rays were normal. At no time during the experiment did the subjects experience middle ear difficulties or have problems with clearing their ears.

As expected, the prolonged confinement period led to some mood fluctuation. There was surprisingly little internal friction, with aggressions being directed toward the food, psychomotor apparatus, and the programmed exercise.

Pre- and postexperimental neurologic and psychiatric evaluations revealed no abnormalities which could be attributed to the subjects participating in the study.

DISCUSSION

Symptoms of mucous membrane dryness and irritation have been noted with previous environmental chamber confinement studies.^{9,11,15} Morgan, et al.,¹¹ studying 8 individuals in 100 per cent oxygen at 190 mm. Hg, described nasal congestion and eye irritation as early manifestations of oxygen toxicity. Herlocher et al., however, evaluating a 100 per cent oxygen, 258 mm. Hg atmosphere attributed the difficulty to the high oxygen flush rate and subsequent decreased relative humidity. With the present work, cracked lips and conjunctivitis appeared to be closely related to periodic declines in water vapor pressure in the chamber.

TABLE III. HEMATOLOGIC STUDIES

Subject Number		Hemoglobin (gm/100 ml)	Hematocrit (%)	White Blood Cells (no./mm ³)	Reticulocytes (%)	Platelets no. x 10 ⁵ mm ³
66	Pre*	14.6±.1	47.8±1.3	8175± 225	0.9±.2	2.78±.02
	Exp**	14.4±.4	45.9±1.0	8638±1402	0.7±.2	2.98±.52
	Post*	13.9±.0	44.9±.1	8250± 350	0.6±.0	3.15±.05
67	Pre	13.6±.3	42.7±.7	7950±2450	1.0±.0	3.40±.70
	Exp	14.1±.5	44.7±1.7	7631±1292	0.8±.2	3.01±.48
	Post	14.1±.3	43.7±.4	8075± 425	0.8±.1	2.93±.08
68	Pre	12.9±.0	41.8±.3	8675± 975	0.9±.1	2.68±.08
	Exp	14.0±.3	44.7±.6	8150±1102	0.8±.2	2.73±.53
	Post	13.4±.5	42.4±.1	8450± 750	0.7±.2	3.33±.13
69	Pre	14.6±.2	45.8±.8	7975±1125	1.1±.1	3.30±.35
	Exp	14.4±.2	45.5±.8	6794±1393	0.9±.3	3.31±.63
	Post	13.8±.2	43.4±.6	6425±1025	0.7±.1	3.65±.30

*Mean values and range for duplicate determinations.

**Mean values and S.D. for 8 determinations.

It is also possible that lithium hydroxide dust from carbon dioxide absorbing canisters contributed to the mucous membrane irritation; although, there is no direct evidence for this. The frequent nasal congestion may have been related to the enriched oxygen atmosphere, even though the oxygen partial pressure of the test environment was similar to sea level values.⁷ Middle ear difficulties, i.e., aural atelectasis or barotitis media, did not occur with the present study. Aural atelectasis was a frequent and persistent finding in long-term studies with 100 per cent oxygen at both 190 and 258 mm. Hg total pressures.^{9,11}

Increased flatus was present continually throughout the hypobaric, oxygen-helium phase. On one occasion, rather severe abdominal pain from trapped intestinal gas occurred in two subjects. At the peak of the symptoms, both individuals were very uncomfortable and subjectively felt they would have had extreme difficulty in performing operational duties. Similar but probably less severe complaints from trapped intestinal gas were noted by Herlocher, et al.,⁹ with 100 per cent oxygen at the same total pressure. It appears that this phenomenon is related to both diet and hypobaric conditions. It can be speculated that helium might aggravate the problem because of decreased absorption of the inert gas from the gastrointestinal tract.

A mild but definite rise of transaminase enzymes was found in subject 67 during the experimental period with no abnormalities of other liver function studies. There were never symptoms of hepatic dysfunction, and postexperimental physical examination was normal. In the light of previous studies with oxygen-helium mixtures at both low¹⁵ and high⁴ atmospheric pressures, it is difficult to relate this enzyme disturbance to the gaseous environment. Adams, et al.,¹ in an earlier report have described the trace contaminants detected during this experiment, and concentrations of the various compounds were considerably below levels known to be toxic for man. This study would appear to eliminate atmospheric contaminants as a cause for the altered serum transaminase pattern. Conceivably, the coincidental occurrence of anicteric viral hepatitis might explain the mild alterations. Cooper, et al.,⁵ found serum glutamic pyruvic transaminase to be the

most sensitive liver function test for the detection of mild anicteric viral hepatitis. They noted sustained elevations of this enzyme (greater than 40 units) in 81 of 3529 Taiwanese and Chinese military personnel.

Averill, et al.,² noted the occurrence of the Wolf-Parkinson-White syndrome in 106 of 67,375 electrocardiograms from flying personnel. In none of these individuals was there evidence of coexisting organic heart disease. The finding of the pre-excitation syndrome on 2 resting electrocardiograms from subject 69 can only be attributed to chance occurrence and unrelated to environmental factors. It is of interest that the pre-excitation pattern was present only during slow resting heart rates and not at faster rates with exercise, when vagal tone is lessened allowing more normal conduction pathways to prevail.

Zalusky, et al.,¹⁴ described a 9.1 per cent reduction in peripheral hematocrit values in 8 individuals living for 30 days in a 100 per cent oxygen, 258 mm. Hg environment. Red cell survival and ferrokinetic studies were normal, and there was no evidence of overt hemolysis. With this 30-day study, conceivably the somewhat increased oxygen partial pressure and the relative inactivity of the confined subjects produced a slight but detectable bone marrow suppression. With the present work, alveolar oxygen partial pressure closely approximated levels at ambient conditions and there was a regular exercise program, both factors tending to obviate any hematologic abnormalities.

Findings of decompression disturbances were absent throughout the entire low pressure, oxygen-helium phase. Dysbarism did not occur despite the vigorous exercise regimen¹⁶ which was initiated 24 hours after ascent to the experimental atmosphere. The exercise loads utilized in this experiment, while not of the duration expected in normal extravehicular maneuvers, equalled or exceeded the level of metabolic energy expected from such activity. This is particularly important with respect to future space programs including those requiring frequent extravehicular maneuvers.

In conclusion, no significant medical abnormalities developed that could be directly attributed to the oxygen-helium, 258 mm. Hg environmental conditions. Four aircrew members tolerated the environment for 56 days without adverse effects. From this study there appears to be no medical contraindication to the use of this atmosphere for future long-term manned space flight.

ACKNOWLEDGMENT

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Study of Man During a 56-day Exposure to an Oxygen-Helium Atmosphere at 258 mm. Hg Total Pressure

XIII. Behavior Factors

CAPT. D. W. RODGIN, USAF, MC, and B. O. HARTMAN, PH.D.

Four officer aircrewmembers performed psychomotor tests 3 times daily and reported on sleep once a day during a 56-day exposure to an oxygen-helium atmosphere at 258 mm. Hg total pressure. Psychomotor and sleep changes were minimal and essentially benign. Adaptation to altered schedules was generally good except for persistent complaints about sleep (not fully supported by sleep histories) from one subject. The tempo of activity and physical exercise regimen apparently prevented behavioral manifestations of deconditioning frequently seen in confinement studies.

formance scores using a test panel containing tasks ranging from simple discrete button pushing to reasonably complex neuromuscular and intellectual functions; (2) oral temperature obtained 3 times each 24 hours; and (3) a self-report sleep experience form filled out once a day. Diaries are an additional data source but have not yet been analyzed.

METHOD AND RESULTS

THE STUDIES of the behavioral effects of simulated space flight have been an integral part of the space medicine program at the School of Aerospace Medicine since its earliest days. Factors such as work-rest ratios, diurnal cycles, work load, speed stress, interpersonal interactions, interpersonal changes, a host of environmental stresses, and loads imposed by personal protective equipment have been investigated. In all such studies, the flight duration with its concomitant confinement, relative isolation and marked alterations from normal schedules have received specific attention. These studies have involved behavioral teams ranging from a single psychologist to a substantial number of psychiatrists and experimental and clinical psychologists. Our role has ranged from that of minimum interference with the more significant physiologic problems to completely co-equal participation. We have found measurable, statistically significant effects in most studies but they have been essentially benign or not qualitatively different from effects found in more conventional working environments. Examples are a speed stress effect compounded by diurnal stress² and interpersonal interactions structured in terms of a mission-oriented, hardware-oriented approach to other crew members.¹ None of these studies have shown statistically significant decrements over time, in flights lasting up to 30 days.

The study to be presented here was carried out by a psychologist and a flight surgeon and was conducted essentially on a minimum interference basis. Three kinds of data were obtained: (1) psychomotor per-

The psychomotor findings will be reported first. The test panel, shown in Figure 1, contains 8 tasks covering 5 broad performance dimensions. These dimensions are vigilance (in four modes), arithmetic, short-term memory, tracking, and problem-solving. These tasks were programmed randomly from a punched tape, with the more complex tasks performed essentially in isolation, and were scored in terms of total response time or error. Subjects operated the test panel for 25 minutes 3 times each day, during the hour before sleep, the hour after sleep, and midway around the clock. Training was initiated prior to entering the test cell and completed prior to going to altitude.

Mechanical malfunctions and unprogrammed equipment modifications resulted in missing data during weeks V through VII. This was partially overcome by separating data into one week units. Table I shows which weekly packages were available for analysis. The data was analyzed by triple classification split-plot

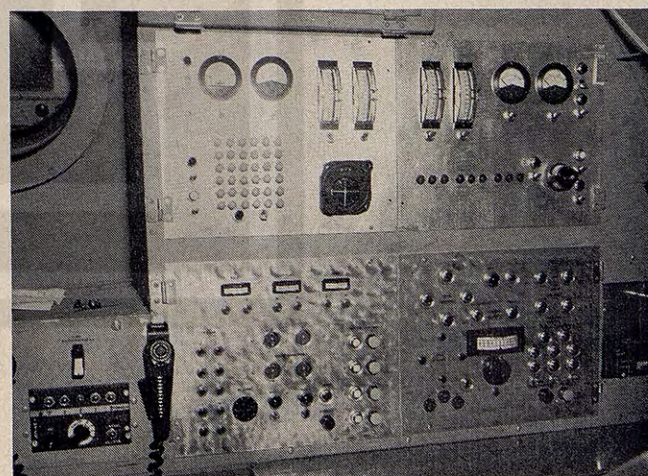


Fig. 1. Photograph of psychomotor test panels located at the forward end of the test cell. Only the bottom pair of panels were operating in this study.

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The research reported in this paper was conducted by personnel of the USAF School of Aerospace Medicine, Aerospace Medical Division, AFSC, United States Air Force, Brooks Air Force Base, Texas. Further reproduction is authorized to satisfy the needs of the U. S. Government.

analyses of variance for each task, with day vs night crews, time of day (adjusted to the sleep schedule), and duration (in weekly packages) as main effects. The results are summarized in Table II. No task yielded a significant F ratio for day-vs-night crew. Subjects sleeping during the external day (1200-2000) did not perform differently than subjects essentially in step with the external schedule. Two of the 4 vigilance tasks approached significance ($P < .10$) for time of day. Though this was short of significance, the means indicated that performance was better during the "mid-day" session (adjusted to the sleep schedules) than during the other two sessions. The F tests performed in this analysis were conventional 2-tailed tests. One could logically argue for a one-tailed test, since there is a considerable body of literature to support a prediction that "mid-day" performance should be better than performance during the other two sessions (before sleep and after awakening). Accordingly, an additional

TABLE I. LISTING OF ONE-WEEK DATA PACKAGES SUITABLE FOR ANALYSIS

Test	Week							
	I	II	III	IV	V	VI	VII	VIII
Arithmetic	X	X	X	X				
Vigilance (meters)	X	X	X	X	X			X
(lights)	X	X	X	X				X
(auditory)	X	X	X	X	X	X	X	X
(lights)	X	X	X	X				X
Tracking	X	X	X	X	X	X	X	X
Short-term memory	X	X	X	X				
Problem solving	X	X	X	X	X	X	X	X

TABLE II. SUMMARY OF MAIN EFFECTS IN THE ANALYSES OF VARIANCE ON THE PSYCHOMOTOR TEST BATTERY AND ON ORAL TEMPERATURE

Test	Crew		Time of Day	Weeks
	Day vs. Nite			
Arithmetic	NS		NS	NS
Vigilance (meters)	NS		NS ($P < .10$)*	NS
(lights)	NS		NS	$P < .005^{**}$
(auditory)	NS		NS ($P < .10$)*	$P < .05$
(lights)	NS		NS	$P < .025$
Tracking	NS		NS	NS
Short-term memory	NS		NS	NS
Problem solving	NS		NS	$P < .001$
Oral temp.	NS		NS	$P < .001$

*Better performance "mid-day."

**Significance interaction (crew x week). Day crew maintained performance but night crew performed considerably poorer in week VIII.

TABLE III. SUMMARY OF ONE-TAILED F TESTS OF DAY-NIGHT CYCLE EFFECTS

Test	Day Crew		Night Crew	
	A.M. vs. Midday	P.M. vs. Midday	A.M. vs. Midday	P.M. vs. Midday
Arithmetic	NS	NS	NS	NS
Vigilance (meters)	$P < .05$	NS	$P < .005$	$P < .025$
(lights)	$P < .05^{*}$	$P < .05^{*}$	NS	NS
(auditory)	NS	NS	NS	$P < .05$
(lights)	NS	NS	NS	NS
Tracking	NS	NS	NS	NS
Short-term memory	NS	NS	$P < .025$	$P < .05$
Problem solving	NS	NS	NS	NS

*Variances not pooled.

series of F ratios were computed, using variances from the previous analysis. Day crews and night crews were analyzed separately. The probability values for these one-tailed F ratios are presented in Table III. Vigilance tasks and short-term memory yielded scattered significant F ratios for the day-night predicted differences. The one-tailed tests did not reveal any systematic day-night differences. In effect, this more detailed analysis supports the tendency toward a diurnal cycle seen in the earlier analyses, but without contributing any additional substance to that finding.

Four tasks yielded significant F ratios for weeks. Examination of weekly means for two of the vigilance tasks showed no meaningful trends over time. On the third vigilance task, the only observable decrement occurred in the nighttime subjects on the final week, when a large (50 per cent) increase in response time was obtained. On the problem solving task, the subjects started with 25 per cent error, dropped to 10 per cent on Week III, and rose systematically to 50 per cent on Week VIII. Two observations argue against the meaningfulness of this decrement. First, the task has low subject acceptance and the changes may reflect only motivational alterations specific to this task. Second, the subjects gave each other unprogrammed problems to solve on the psychomotor panel during the middle period at altitude and they successfully solved these problems at a time when the formal problem-solving measure was showing a systematic decline.

Oral temperatures were obtained with a rectal probe inserted for one minute at the end of each psychomotor test period using a Yellow Spring Instrument Company Telethermometer device for readout. An analysis of variance like that described above yielded a significant F ratio only for "weeks." The values rose systematically from 97.6 to 98.2 from Week I through Week VIII.

Sleep histories were obtained, as indicated earlier, with a self-reporting form filled out daily. The reporting form is shown in Figure 2. A mean sleep duration was computed for each subject during the pre-altitude ground-level period, for each week, and for the post-altitude ground-level period. These values, plus a baseline value obtained several months earlier, are shown in Table IV. In general, all 4 subjects slept nearly all of the 8 hours allotted to them each night. Subject 67

TABLE IV. MEAN DURATION OF SLEEP IN HOURS ON A SUBJECT-BY-SUBJECT BASIS USING A SELF-REPORTING TECHNIQUE. MOST VALUES ARE THE AVERAGE OF ONE WEEK.

Period	Subjects			
	66	67	68	69
Baseline*	7.8	7.8	7.3	8.1
Ground level	7.1	7.4	7.5	6.6
Week I	7.5	7.6	7.9	6.3
II	8.4	8.0	7.8	8.5
III	8.2	7.6	7.8	8.8
IV	8.0	7.9	8.1	7.4
V	8.1	7.6	8.4	7.9
VI	8.1	7.6	8.2	6.9
VII	8.1	7.4	8.1	7.4
VIII	8.2	6.5	8.4	7.3
Ground level	7.6	6.3	7.1	6.4

*Obtained from a 10-day period several months earlier in another program.

Fig. 2. Photograph of the self-reporting sleep form, presented to indicate the kinds of questions asked and the kind of report required. These forms were filled out daily and passed out through the lock.

SLEEP SURVEY										DATE	
NAME (Last, First, MI)				GRADE	SERIAL NR	AGE	PRIMARY DUTY				
1. On the chart below, mark an X for each half hour to indicate how much you slept in the past 24 hours. Now use the chart to show how well you slept. Use 1 to show drowsy or light sleep, 2 to show moderate or average sleep, and 3 to show deep sleep. Write these numbers below the X's.											
DAYTIME											
HOW MUCH											
HOW WELL											
	0600	0700	0800	0900	1000	1100	1200 (Noon)	1300	1400	1500	1600
NIGHTTIME											
HOW MUCH											
HOW WELL											
	1800	1900	2000	2100	2200	2300	2400 (Midnight)	0100	0200	0300	0400
2. HOW MUCH TROUBLE DID YOU HAVE GOING TO SLEEP LAST NIGHT?						3. HOW MANY TIMES DID YOU WAKE UP LAST NIGHT?					
☐ NONE ☐ SLIGHT ☐ MODERATE ☐ CONSIDERABLE						☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10					
4. HOW RESTED DO YOU FEEL?						5. DO YOU FEEL LIKE YOU COULD HAVE USED SOME MORE SLEEP?					
☐ WELL RESTED ☐ MODERATELY RESTED ☐ SLIGHTLY RESTED ☐ NOT AT ALL						☐ YES ☐ NO					
6. HOW MUCH DID YOU DREAM LAST NIGHT?						7. HOW MANY DREAMS DID YOU HAVE?					
☐ NONE ☐ SLIGHT ☐ MODERATE ☐ CONSIDERABLE						☐ 0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10					
REMARKS											

tapered off during the second month at altitude. Subject 69 showed wider swings from week to week, with notably less sleep early in the study despite the fact that his baseline value was the highest of the 4. In response to specific questions on the form, three subjects reported modally that they felt well-rested (Table V). Subject 69's mode was "moderately well-rested." Three subjects reported modally that they felt that they did not need more sleep. Subject 69 reported modally that he felt he needed more sleep.

The sleep reporting form requires not only that the subject report time asleep, but also an estimate of the depth of sleep (light, moderate, or deep) for each half-hour block. This is, of course, a more difficult kind of report to make but the interest in sleep in space suggests that these reports should be summarized. Table VI presents percentages of half-hour blocks in each of the 3 stages for each subject. Most values are based on one-week blocks.

Subject 66 reported deep sleep modally prior to the study and continued to report deep sleep modally throughout. Subject 67 reported moderate sleep modally prior to the study, shifted to deep sleep modally for the first half of the study, and apparently started to shift back toward a mode of moderate sleep during the last half. Subject 68 reported moderate sleep modally prior to the study, shifted to a mode of deep sleep and remained there during the study. Subject 69 reported deep sleep modally prior to the study, shifted to moderate sleep, then to deep sleep, and finally back to moderate sleep as the mode, with considerably higher percentages of light sleep than that reported by the other subjects. It appears that Subject 69's sleep problem was in depth of sleep rather than sleep duration. Subject 67 shows a somewhat similar distribution for the latter half of the study, but did not regard it as a problem, according to his answers on questions 4 and 5 of the survey form (see Table V). It is of interest that Subject 69 reported feeling well rested frequently during weeks VII and VIII when he was clearly in moderate sleep modally.

DISCUSSION

In general, the data which we obtained are consistent with the following conclusions. From a gross behavioral point of view:

1. Changes during the 56 days were minimal and essentially benign:

- Only one psychomotor task (problem solving) yielded a *systematic* decrement over time; the difficulties in evaluating this finding meaningfully

TABLE V. FREQUENCY DISTRIBUTION FOR EACH ANSWER IN QUESTIONS 4 AND 5 OF THE SLEEP SURVEY CONCERNING FEELINGS ABOUT THE ADEQUACY OF SLEEP

Subjects	How rested do you feel?				Do you need more sleep?	
	Well Rested	Mod. Rested	Sltly. Rested	Not Rested	Yes	No
66	53*	17	0	0	28	42*
67	62*	7	1	0	8	62*
68	53*	15	1	1	15	55*
69	9	58*	1	2	64*	6

*Predominant report.

TABLE VI. PERCENTAGE OF HALF HOUR BLOCKS IN EACH OF 3 SLEEP STAGES ON THE BASIS OF SELF-REPORTS (SLEEP SURVEY FORM). MOST VALUES ARE BASED ON ONE WEEK.

Period	Subjects											
	66			67			68			69		
	1	2	3	1	2	3	1	2	3	1	2	3
Baseline	0	9	88	17	82	0	9	87	6	13	18	69
Ground level	8	8	84	13	19	68	3	7	90	37	40	23
Week I	8	13	79	20	24	56	3	12	85	31	40	29
II	9	11	80	13	22	63	6	4	90	18	24	58
III	16	14	70	15	15	70	3	4	93	16	33	51
IV	0	9	91	16	12	70	11	9	80	20	49	31
V	0	9	91	21	17	62	5	4	91	28	59	13
VI	0	4	96	22	29	49	0	7	93	31	64	5
VII	1	11	88	30	26	44	2	13	85	27	73	0
VIII	5	10	85	29	26	46	8	8	84	43	57	0
Ground level	7	9	84	14	23	63	2	0	98	35	65	0

- have already been discussed.
- b. Sleep histories were essentially normal except for one subject who experienced persistent difficulties, though he did in fact spend a substantial part of the programmed sleep period asleep and accomplished all of the work assigned to him.
 - c. The observed effectiveness with which in-chamber physiologic procedures were carried out improved week by week.
2. No significant problems in adjusting to the altered schedules were seen:
- a. This statement must be tempered by the persistent sleeping problems experienced by one subject, which might reflect poor adaptation in this area.
 - b. It must also be tempered by our failure to obtain a substantial "time-of-day" effect in psychomotor and oral temperature data.
3. The physical exercise regimen and tempo of activity in general were sufficient to prevent deconditioning (as reflected in behavioral measures) frequently seen in confinement studies:
- a. Subjects slept for essentially normal durations (just under 8 hours on the average) and modally re-

ported feeling well-rested or moderately well-rested.

Changes in emotional tone are a separate question—one on which analyses of the diaries which the subjects kept during the experiment will shed some light. Persistent complaints about sleep by one subject are out of proportion to the ratings he submitted daily. Observations by the staff of interpersonal aggression, hostility toward people outside the test cell, and feelings specific to psychomotor testing indicate that changes in emotional tone occurred during the test. The relevance of these changes to actual space flight may be difficult to calibrate, and will require detailed study and quantification of diaries from this and earlier simulated space flights.

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Emphasizing Physical Principles in Biological Research

**A Review of the Applications of
Solid State Physics Concepts to
Biological Systems*†**

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Review of biological
superconduction - pages 6-9

UNIVERSITY PUBLICATIONS

A Review of the Applications of Solid State Physics Concepts to Biological Systems*†

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ABSTRACT. The evidence for solid state physical processes in diverse biological systems is reviewed. Semiconduction of electrons across the enzyme particles as the rate-limiting process in cytochrome oxidase is evidenced by the peculiar kinetic patterns of this enzyme and by microwave Hall effect measurements. PN junction conduction of electrons is suggested by kinetics of photobiological free radicals in eye and photosynthesis. Superconduction at physiological temperatures may be involved in growth and nerve. Phonons and polarons seem likely to be involved in mitochondrial phosphorylation. Piezoelectricity and pyroelectricity may be involved in growth and nerve. Infrared electromagnetic waves may transmit energy in lipid bilayers of nerve and mitochondria. Complexed sodium and potassium ions in structured cell water may be analogous to valence band electrons in a semiconductor, and the free cations may be considered analogous to conduction band electrons. Ionic processes in cell water therefore resemble electronic conduction processes in solid semiconductors, which leads to kinetic predictions in agreement with experiment. The future of solid state biology depends on the development of new experimental methods able to measure solid state physical properties in biological materials which are non-crystalline, impure, particulate, and wet.

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*Dedicated to Felix Gutmann, who stimulated me to write this, and whose book on organic solid state physics provides a foundation for biological solid state physics.

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I. Introduction

The physics and chemistry of the *liquid* state has been widely applied to biological systems. However in addition to liquids, cells contain *solids* (particles and membranes), so that it is reasonable to expect that events of biological importance might occur in the solid parts of cells, and that these events should be governed by the physics of solids. Only recently, and only in a few laboratories, has serious attention been given to this possibility.

Szent-Györgyi¹ in 1941 suggested a role for semiconduction in biology, but the high activation energies of semiconduction in many biological solids measured during subsequent years made this suggestion seem unlikely. However, a particular protein (cytochrome oxidase), which gives kinetic evidence of semiconduction, was measured to have a much lower semiconduction activation energy, approximately equal to the activation energy of the enzymatic reaction catalyzed by this protein, which supports the concept of a semiconductive mechanism. Evidence for other sorts of solid state physical processes in cells (P-N junction conduction, superconduction, piezoelectricity, infrared electromagnetic wave transmission) has also been developed in recent years.

The only previous comprehensive reviews of solid state biology were those of Rosenberg and Postow² in 1969 and Cope³ in 1970. Much has been done since then. The present review will update the 1970 review of Cope.³ We shall describe in considerable detail work appearing since 1970, but earlier work will be described less completely, and the previous review³ should be consulted if more details are needed regarding earlier studies. For general background in theory and experiment of organic semiconduction, the books by Gutman and Lyons⁴ and Boguslavskii and Vannekov⁵ may be consulted.

Solid state biology has been approached in two different ways from two opposite ends of the problem. The two approaches are described in partial separation in the next two sections of this review.

First, purified organic solids, extracted from living animals or plants, have been tested for various solid state physical properties such as semiconduction, superconduction and piezoelectricity. These and other solid state physical phenomena have indeed been observed in purified biological solids. One does not immediately know, however, whether the solid state physical properties of isolated, purified biological solids necessarily play any role in the function of living systems. They might not.

Second, solid state biology has been approached from the other end. Some observed properties of *organized living systems* are not easy to explain in terms of chemical processes in aqueous solutions, but may be derived readily from hypotheses of solid state physical processes in cells. One may then use the observed characteristics of organized living systems to deduce the quantitative properties of solid state physical phenomena which may cause them. One may then compare these deductions with the observed solid state physical properties of purified components of these complex organized living systems.

II. Solid State Physical Phenomena in Components of Living Cells

A. Semiconduction. In 1941, Szent-Györgyi¹ hypothesized that semiconduction of electrons in solid proteins or DNA might play a role in biological function. This idea led many investigators to measure the temperature dependence of electron conductivity for dried, compressed pellets of many components and extracts of biological systems, such as proteins or cell fragments. In general, the data did indeed conform to the semiconduction equation

$$\sigma = \sigma_0 \exp(-E_a/kT) \quad (1)$$

where σ = measured conductivity, E_a is activation energy of semiconduction, k is Boltzmann constant, T is absolute temperature and σ_0 is a constant. However, the value of E_a was usually in the region of 1.0 ev, so that conductivity at physiological temperature was very low, perhaps too low to

serve any useful biological function, and the 1.0 ev was much higher than the observed activation energies of most biological processes.⁶ For these reasons, by the middle 1950's, most investigators concluded that semiconduction probably played no role in biology.

Several important points had however been overlooked.

First, the possible influence of water was overlooked. Measurements of semiconduction had been made only in *dried* systems. Living systems function only when wet (80 percent water). Water as an impurity at the surface of tiny biological particles or membranes might change electron conductivity by many orders of magnitude, as impurities adsorbed on thin film inorganic semiconductors are often observed to do. This effect of water adsorbed on a thin film semiconductor is used for humidity measurement in a commercially available humidity meter. The experiments of Rosenberg^{7,8} suggest that the presence of water may indeed cause significant changes in conductivity and activation energy of semiconduction of proteins. A pulsed electron beam method with wet protein films in Russia seems to show the same result.^{5,9}

Second, it was overlooked that a few proteins might exist which had unusual structures, specially adapted for high electron conductivities and low semiconduction activation energies. Cope¹⁰ showed that the enzyme cytochrome oxidase had kinetic properties which suggested that it was rate-limited by solid state conduction in this particular protein (which has a specific and complicated structure quite different from most proteins). Then Straub^{11,12} measured semiconduction activation energy in this particular protein, cytochrome oxidase, and found it to be approximately 0.3 ev (3 times lower than with most proteins, the high values of which were confirmed by Straub). It therefore seems that although most proteins do indeed have very high values of semiconduction activation energy which make them poorly suited to perform biological functions by semiconduction, there is at least one specialized protein with a low activation energy well suited to the biochemical role which it seems to play.

Third, it was overlooked that electron conduction *within* individual solid particles might be quite different from conduction across interfaces from particle to particle, which may be what one measures when one puts electrodes across a compressed mass of thousands of microscopic particles. To measure electron conductivity *within* individual tiny biological particles, a microwave Hall effect technique was developed by Trukhan^{13,14} in Russia, and applied to various biological particles by various investigators.¹⁴⁻²² The results seem to show that electron conductivities within individual biological particles may be much higher than previously deduced from conductivities of compressed masses of particles, and that cytochrome oxidase activity is positively correlated with microwave Hall effect mobility of electrons.^{19,20}

It therefore seems likely that semiconduction within solid biological particles may indeed play a role in biological function in at least a few important biological systems, especially in the enzyme cytochrome oxidase. For more details and references to the earlier work, the previous review³ may be consulted.*

B. PN Junction or Activation Energy Barrier Conduction. Conduction across an activation energy barrier, such as at a PN solid-state junction or liquid-solid junction of an electrode in an aqueous solution commonly shows a exponential or logarithmic voltage-current equation.

In exponential form

$$I = I_0 \exp(V/kT) \quad (2)$$

is known as the diode equation. In logarithmic form

$$V = a + b \log(I) \quad (3)$$

is known as the Tafel equation of electrodes, where V is voltage, I is current, k is the Boltzmann constant, T is absolute temperature and a and b are constants.

Liquid-solid, and solid-solid interfaces are common in biological systems. Therefore, this type of conduction might be expected in biological systems. Its existence has been deduced from behavior of organized systems as described in Section III of this review.

C. Phonons and Polarons. It has been proposed by Straub^{11,23,24} that phonons (packets of lattice vibrational energy) may be involved in transmission of energy from oxidative reactions to phosphorylative reactions in solid protein systems such as membranes of mitochondria. Destruction of the phonons in the solid protein by thermalization may be prevented by the hydrophobic bonding of a layer of lipid separating the protein from the surrounding water.²⁴ The mitochondrial processes of respiratory control, reversed electron flow, uncoupling, and utilization of two electron transfer to accomplish one bond formation seem to be compatible with the phonon hypothesis.^{11,23-26}

Phonon coupling between a pair of electrons to form a superconductive Cooper pair may also occur in biological systems at physiological temperatures. Biological superconduction will be discussed in the next section of this review.

A problem with the concept of phonons in mitochondria is probable lack of mobility, because these phonons would need to have an energy of approximately 0.39 eV (9.0 kcal/mole) which is the energy required for phosphorylation of ADP (the chief phosphorylation reaction carried out by mitochondria). Phonons of this energy are *optical* phonons (wavelength

* Good experimental evidence for electronic conduction in lipid membranes¹⁶⁷⁻¹⁶⁹ and across the shells of crabs¹⁷⁰⁻¹⁷² is also available.

3.24 microns in the infrared band). Optical phonons are generally immobile, so that transport of energy by phonons from sites of oxidation to sites of phosphorylation might be expected to be slow.²⁵ However, the structure and geometry of the lipid bilayer of mitochondria seems well adapted to carry infrared electromagnetic waves (photons), which could serve as a rapid and efficient method to carry the energy of the phonons throughout the mitochondrion.²⁵ This mechanism is discussed in Section IIIC of this review.

That polarons rather than phonons may be the vibrational packets in mitochondria is suggested by the reversal at low temperatures of the enzyme activity vs $(1/T)$ curve of cytochrome oxidase measured by DeVault and Chance²⁷ as analyzed by Cope and Straub.¹² Such a reversal is predicted from the concept that the charge carriers in solid cytochrome oxidase are polarons, according to the theory of Holstein.^{28,29} A polaron is an electron or hole tied to a phonon. The probability of polaron conduction in organic semiconductors has been discussed by Siebrand³⁰ and by Gutman and Lyons.⁴

It has been suggested by Kemeny and Rosenberg¹⁵⁹ that polarons may be the charge carriers in proteins and other organic semiconductors, and may be responsible for the observed compensation law in these substances.

Volkenstein¹⁶⁰ has proposed a state in a protein which he calls a conformon, which he claims should be different from a polaron because of the non-regularity of the atomic lattice, resulting in lack of mobility and short lifetime. This has been studied further by others.¹⁶⁵⁻¹⁶⁶

It has been proposed on theoretical grounds that melanin (the black pigment found in skin, eye, and in parts of the brain and ear) may be a particularly efficient converter of photons to phonons and of the reverse process.¹⁶¹⁻¹⁶² Evidence suggesting the phonon to photon conversion by melanin was the observation that ultrasound (10^4 Hz) was more damaging to cells containing melanin than to similar cells without melanin, correlated with previous observations that photons of high intensity light are more damaging to cells if melanin is present.¹⁶³⁻¹⁶⁴ At lower intensities, however, melanin protects cells against damage by light.¹⁶²

D. Superconduction. Superconduction is the conduction of electrons without the generation of heat, and hence with an electrical resistance equal to zero. Until recently, superconduction had been observed only in certain metals and only at a temperature colder than approximately 20° Kelvin. Theorists have long predicted that superconduction might occur at room temperature in organic solids. The development of new techniques of very high sensitivity has now led to the experimental detection of superconductive transitions in organic solids (various cholates) at temperatures as high as

277°K. Indirect evidence suggests that superconduction plays a controlling role in various functions of living systems at physiological temperatures.

A comprehensive mathematical theory (the BCS theory) of superconduction has been developed which explains and predicts many (but not all) of the experimentally observed electrical and magnetic properties of superconductors³¹ and properties of electron tunneling across junctions between superconductors.^{32,33} An extension of the BCS theory by Little^{34,35} predicted that superconduction should occur in certain types of organic polymers *at room temperatures and above*. There is much controversy among theoretical physicists regarding the validity of this prediction.³⁶ All attempts to synthesize Little's superconductive polymers have so far failed, which may be due to inadequacies of the attempts.

Pauling³⁷ and London³⁸ claim that electron currents around benzene rings in aromatic compounds are analogous to superconductive currents in metals. This claim is disputed by Musher.^{39,40} However, much quantitative data on magnitudes of chemical shifts of observed nuclear magnetic resonance spectra of compounds containing benzene rings is explainable in terms of superconductive ring currents.⁴¹⁻⁴⁵

High temperature superconduction has also been predicted in a thin metal film adjacent to a dielectric layer, where the interaction between the two electrons of the pair occurs through the dielectric instead of within the conductor.^{36,46-48} All attempts to manufacture such superconductive bilayers or sandwiches have failed, perhaps because the correct method was not tried.

Experimental evidence of superconduction in organic solids at high temperatures has been obtained very recently by Halpern and Wolf.⁴⁹⁻⁵¹ These findings required the use of new instrumental methods for the measurement of extremely small changes in magnetic susceptibility.^{49-51,177} Transitions (abrupt changes) in susceptibility as a function of temperature and of applied magnetic field were observed in various cholates (4 rings with various side chains).⁴⁹⁻⁵¹ The rise of susceptibility is predicated on the concept that transition from normal to superconducting regions would initiate the Meissner effect which would cause internal magnetic field changes to be less than those imposed externally. X-ray diffraction patterns were observed to be unchanged at temperatures across the transition temperature, which showed that no change occurred in the structure of the atomic lattice.⁵² Therefore, the transition must be due to *electronic* rearrangements. A change between superconducting and normal conducting state was the most obvious possibility, and was consistent with the experimental data.^{49-51,177} Transition temperatures observed for the cholates were as high as 277°K. Other types of organic compounds studied did not show evidence of superconductivity.^{50,51} Cholates showed superconductive phenomena only when crystallized in

certain ways.^{50-51,177} The heights of the observed transitions were small, which indicated that only a small fraction of cholate molecules were in the superconducting state at any given time.

Extrapolation⁵² of the experimental data on cholates^{49-51,177} suggests that cholesterol (a major constituent of nerve and of other tissues) may superconduct at physiological temperatures and above, so that cholesterol might be a site of superconduction in living systems.

Another experimental observation of probable high temperature organic superconduction is the recent data of Antonowicz.⁵⁴ He observed fluctuations in current through thin carbon films at room temperature as a function of magnetic field at very low fields (0.5 to 2.0 Gauss), with a pattern like that seen with the Josephson effect for two-electron tunneling at superconducting junctions. No other known physical phenomenon seems adequate to explain this experimental finding.

There is evidence suggesting that high electric fields may promote superconduction in organic fields at high temperatures, and that this might play a role in nerve conduction.⁵³ The room temperature Josephson effect of Antonowicz⁵⁴ in carbon films required a 2 volt electric potential across a carbon film that was presumably only a few molecules thick, so that the electric field would be very high. Magnetic fields of 2000-3000 Gauss cause changes in photocurrent in organic solids (anthracene and tetracene) at room temperature but only if the voltage is sufficiently high to be above the ohmic conduction region and into the square law conduction region.⁵⁵ This probably implies charge injection into the organic crystal.^{53,55} The magnetic effects in both the above sets of experiments, as well as the square law conduction, may be understood from the hypothesis that high voltage electron injection into organic solids may produce superconductive Cooper pair charge carriers.⁵³ It is reasonable to consider that this superconductive process might occur in the thin lipid layers of nerve in response to the high electric fields which occur there,⁵³ especially since extrapolation of the data on cholates suggests that cholesterol (which occurs in large quantities in nerve) should superconduct at physiological temperatures.⁵²

The evidence for high temperature superconduction in organic solids raises the possibility that superconduction in solid portions of cells might play a role in living systems. Three lines of indirect evidence suggest that this is so.

First, that bulk superconduction may play a biological role is suggested by the abrupt changes in rates of metabolism of growing tissues induced by magnetic fields of 50 to 150 Gauss,⁵⁶⁻⁵⁸ although lower fields produced no effect, the higher fields produced no additional effect. A superconductive transition is the most obvious possible cause of a discontinuous effect produced by a magnetic field.⁵⁶

Second, a role in biology for *two*-electron superconductive tunneling (Josephson effect) is suggested by the demonstration that numerous living systems (birds, insects, snails, and possibly man) can detect very low magnetic fields (0.5 to 1.0 Gauss).^{59,60} No physical phenomenon other than the Josephson effect seems to have sufficiently high magnetic sensitivity to provide the physical basis for this observed biological magnetodetection.^{59,60} In addition, the rates of some biological processes are observed to show *negative* temperature coefficients, which cannot be explained by ordinary chemical and physical rate processes, which all show *positive* temperature coefficients. However, Josephson superconductive tunneling has a *negative* temperature coefficient and hence could provide a physical basis for these observed biological phenomena.⁵⁶

Third, a controlling role for *single*-electron superconductive tunneling in living systems is suggested by the temperature dependences of the rates of certain nerve and growth processes.⁵⁶ For the rates of most biological processes, like most chemical processes, the temperature dependence is given by linear plots of log of rate vs $1/T$ (a linear Arrhenius plot). However, a few biological processes show curved Arrhenius plots, and some of these are described by the equation

$$E_a^2 = aT + b \quad (4)$$

where E_a is the activation energy, measured from the slope of the Arrhenius plot, T is temperature in degrees Kelvin and a and b are constants. This type of temperature dependence is predicted for and observed in *single*-electron superconductive tunneling.⁵⁶ Therefore, one may surmise that these biological processes (all involving either nerve or growth) are rate-limited by single-electron superconductive tunneling.⁵⁶

E. Piezoelectricity and Pyroelectricity. A piezoelectric solid is usually defined as one which generates a voltage proportional to an applied mechanical force, due to mechanical distortion or displacement of electric dipoles in the lattice.⁶¹ Piezoelectricity has been observed directly in bone^{62,63} and wood⁶⁴ by measuring electrical potentials induced by bending or compression.

Biological materials which are available only in powdered form cannot be tested for piezoelectricity by the *direct* method used for bone and wood. However, an *indirect* method capable of measuring weak piezoelectricity in powders is available and has been applied to dry biological powders. This method was developed by Giebe and Sheibe⁶⁵ and has been improved by others.⁶⁶⁻⁷⁰ It uses the principle of the vacuum tube crystal oscillator, which has for many years been used as a source of frequency-stable radio-frequency voltage. In this circuit, a piezoelectric quartz crystal, cut to a size and shape to allow mechanical vibration resonance at the desired radiofrequency, is placed between metal electrodes, which are connected

to a vacuum tube feedback circuit. The entire electro-mechanical system then goes into oscillation, which generates a sinusoidal voltage whose frequency is determined by the mechanical properties of the piezoelectric crystal.

Because electrical oscillation in this type of circuit depends on the piezoelectricity of the crystal, the electrical oscillations can be used to detect piezoelectricity in the crystal. The same may be done if a compacted powder is used instead of a single crystal.

To obtain highest sensitivity of detection of weak piezoelectricity in powdered materials, special superregenerative oscillator circuits have been developed in which the amplitude of oscillation is decreased at frequencies at which piezoelectric resonance occurs in the powder.⁶⁵⁻⁷⁰ These circuits have been used to measure the piezoelectric spectrum of biological materials as a function of frequency and temperature by Duchesne and Monfils^{69,71-73} and others.^{70,74-77}

Duchesne and Monfils^{69,71-73} observed that the positions of the peaks in the piezoelectric spectrum showed a temperature dependence which could be described by the equation

$$\Delta = \frac{-1}{f} \frac{df}{dT} \quad (4A)$$

where df/dT is the observed change in the frequency (f) of the piezoelectric absorption peak per unit change in temperature (T), f is the approximate frequency of the peak, and the calculated constant (Δ) turns out to have approximately the same value for all peaks at all frequencies for any particular type of powdered sample. Δ is therefore a measurable piezoelectric constant characteristic of each organic or biological powder. Duchesne *et al*^{69,71-73} believe that the constant Δ is related to other physical parameters of the solid by equation

$$\Delta = \alpha\gamma \quad (4B)$$

where α is the thermal expansion coefficient of the solid sample, and γ is the Grüneisen constant,^{78,79} which is a dimensionless measure of the change in entropy with volume of the crystal.

Measured values of Δ for various biological and non-biological powders are given in Table 1.

We should like to know whether the observed piezoelectric properties of these biological materials play any role in function of living systems. They might or might not.

The characteristics of several biological processes suggest that piezoelectric phenomena do indeed perform biological functions.

First, it is observed that the architecture of bone continuously adapts itself to the mechanical loads placed upon it.⁸⁰ Growth of bone is

TABLE 1
Piezoelectric Temperature Coefficient of Various Substances

Sample	$\Delta (\times 10^{-6}) (^{\circ}\text{C})^{-1}$	Reference
Quartz	6	77
Zinc sulfide	46	77
Potassium sodium tartrate	400	77
1-Chloramphenicol	100	71
DNA (thymus)	50	73
DNA (sperm)	56	73
DNA (yeast)	18	77
RNA (yeast)	6	77
Myosin	85	73
Actomyosin	100	73
Collagen	47	73

The constant Δ is characteristic of the material and is calculated by equation 4A from measurements of the frequency shifts of piezoelectric resonance absorption peaks as a function of temperature, which are measured as described in the text.

experimentally observed to be controllable by applied electric potentials.^{81,83-88} Piezoelectric potentials of bone, generated in response to mechanical stress on the bone, may then serve to control the growth of bone to adapt it to the stresses placed upon it.⁸²

Second, growth of tissue is stimulated by implantation of piezoelectric dusts.⁸⁹⁻⁹¹

Third, the temperature dependence of the resting potential of nerve is observed to conform to the temperature dependence of piezoelectric resonance.⁹² Petrov⁹³ has theorized that a membrane potential may be dependent on piezoelectric (flexoelectric) properties of liquid crystalline lipids. Piezoelectric properties of liquid crystals have been studied theoretically⁹⁴⁻⁹⁷ and experimentally.^{98,99} Therefore, piezoelectricity may be involved in nerve function.

Fourth, some of the observed properties of muscle stretch receptors may be derived from the hypothesis that piezoelectric elastic polymers are involved in detection of muscle stretch.¹⁰⁰

Piezoelectric materials are usually also pyroelectric, which means that an electric potential is generated by the application of heat or cold. Pyroelectricity has been measured directly in tendon and bone,¹⁴⁹⁻¹⁵⁵ meninges,¹⁵⁶ spinal cord,¹⁴⁹ and nerves.¹⁵⁶ Also, it is observed that the electrical responses of temperature sense organs of living systems show behavior which is typical of pyroelectric materials.¹⁰⁰ Application of a square pulse of increased temperature causes transient nerve responses of opposite sign at the two ends of the temperature pulse¹⁰⁰ (Fig. 1). This suggests that pyroelectricity does indeed play a role in biological temperature detection.

F. Amorphous Semiconductor Switching. McGinness *et al*¹⁵⁷ have shown that melanins (from melanoma tumors or synthetic melanins) switch from low current to high current when voltage is increased past a certain threshold voltage, which resembles the switching behavior of inorganic amorphous semiconductors (Ovshinsky effect). The increase from low to high current is 10 to 1000 times.

The mechanism of this effect in inorganic materials is in dispute, so that extrapolations of mechanism from inorganics to melanin have not been made. Some information regarding the mechanism of switching in melanin has been deduced from the experiments of McGinness *et al*.¹⁵⁷

First, the current in the on-state is mostly electronic rather than ionic, because weight loss of sample which would occur if water were lost by electrolysis is negligible.¹⁵⁷

Second, even though conduction is electronic, water is necessary for switching, because thoroughly dried melanin does not switch, but switching ability is recovered when the melanin is slightly hydrated.¹⁵⁷ Perhaps, water alters electronic conductivity of the melanin by lowering the activation energy for conduction of electrons between sites, analogous to the mechanism proposed by Rosenberg⁸ for proteins. This requirement for water for switching to the high conductivity state may be related to the requirement for water for reversibility of electron transfer measured by generation and decay of photogenerated free radicals in eye melanin.¹⁵⁸

The voltage threshold for switching for hydrated melanin is approximately 500 volts/cm which is much lower than the 5×10^5 volts/cm observed for cytochrome C, which is comparable to the values of voltage threshold observed for inorganics.¹⁵⁷ Various other organic materials of biological origin were tested and found not to show switching behavior.¹⁵⁷

It would be interesting to know whether switching behavior in melanin plays any role in biological switching processes in regions where melanin occurs, such as skin, retina, brain, or ear.

III. Solid State Processes in Integrated Biological Systems

A. Growth. It is experimentally observable that a living bone grows continuously in a way that adapts it to withstand the stresses to which it is subjected.⁸⁰ The boney struts on the inside of a long bone (e.g. the leg bone) are located along the lines of stress. During life, a bone may change its structure to increase or decrease its strength to adapt to the loads habitually placed upon it. For example, a longshoreman's bones

TRANSIENT BIOLOGICAL RESPONSE TO TEMPERATURE

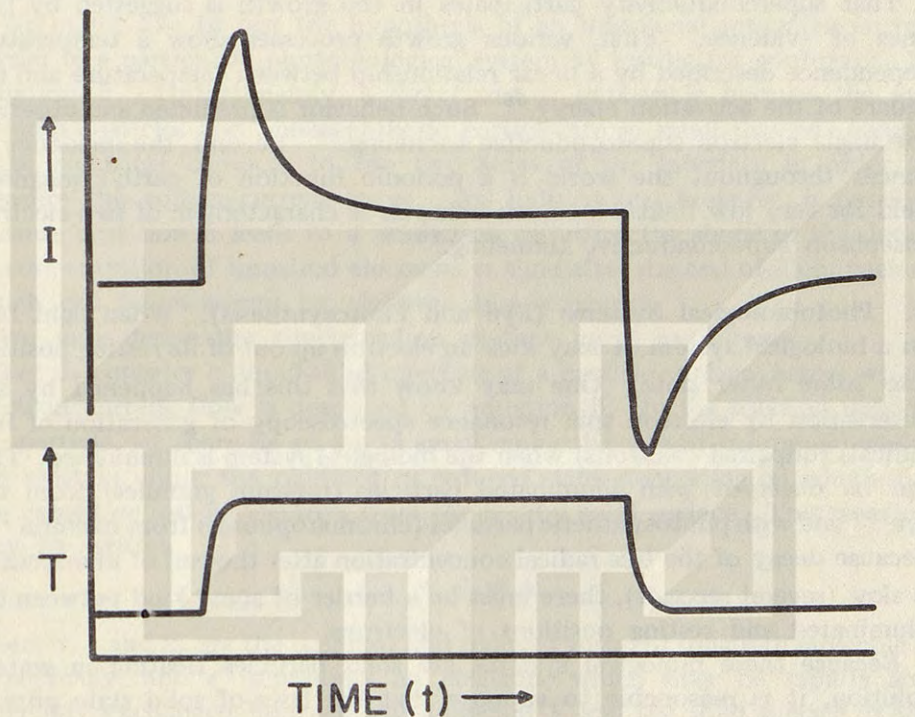


Fig. 1. Transient Biological Response to Temperature Pulse. Lower curve shows a pulse of temperature (T) applied to biological system during the passage of time (t). Upper curve sketches the form of a typical pattern of biological response (I), with transients of opposite sign at the start and end of the temperature pulse, and a steady state response after the initial transient has faded away.¹⁰⁰

will become much stronger than average, while a person who remains in bed for some months will develop lighter bones. There must therefore exist some sort of system which controls the growth of bones in response to applied stress. It has been observed that application of a D.C. electric potential to the end of a broken bone causes growth.^{81,83-88} It has also been observed that bones are piezoelectric, i.e. bending a bone generates an electrical potential.^{62,63} It was therefore suggested by Becker, Bassett and Bachman⁸² that these two phenomena working together might serve as a closed-loop feedback system by which the body controls the growth of bones in response to the applied stresses.

An additional indication of a relation of piezoelectricity to growth is the observation that implantation of piezoelectric dusts stimulates tissue growth.⁸⁹⁻⁹¹

That superconductivity participates in cell growth is suggested by two lines of evidence. First, various growth processes show a temperature dependence described by a linear relationship between temperature and the square of the activation energy.⁵⁶ Such behavior is predicted and observed for single electron superconductive tunneling.⁵⁶ Second, the incidence of cancer throughout the world is a periodic function of earth's magnetic field for very low fields.¹⁰¹ Such behavior is characteristic of two electron Josephson superconductive tunneling.¹⁰¹

B. Photobiological Systems (Eye and Photosynthesis). When light falls on a biological system, it may kick an electron up out of its resting position into some other state. One may know that this has happened by the observation by electron spin resonance spectroscopy of generation of free radicals (unpaired electrons) when the biological system is illuminated. This can be observed with illuminated particles (melanin granules) from the eye¹⁰² and with photosynthetic particles (chromatophores) from bacteria.¹⁰³ Because decay of the free radical concentration after the end of illumination is slow (several seconds), there must be a barrier of some kind between the illuminated and resting positions of electrons.

Because these biological systems are solid particles floating in watery solution, it is reasonable to suppose that the laws of solid state physics apply within the particle, and that the particle surface acts as a solid-liquid interface, to which electrode physics should apply.

A simple hypothesis for such a system as to why return of illuminated electrons to equilibrium might be slow is that light has kicked the electrons across an activation energy barrier at the particle surface. Activation energy barriers at liquid-solid interfaces are commonly observed¹⁰⁵⁻¹⁰⁷ in electrode physics, and give rise to a voltage-current relation across the interface of the form

$$V = b - \frac{RT}{Fa} \log_e(-i) \quad (5)$$

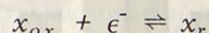
where V is voltage across the interface, i is current across the interface, T is absolute temperature, R is the gas constant, and a and b are constants. In electrode physics, this relationship is known as the Tafel equation,^{104,105} but the same relation (written in exponential form) is seen in solid state physics at solid-solid interfaces, at which it is known as the diode equation. The use of equation 5 for electron conduction across interfaces is well justified on the basis of experimental observation. It also has a theoretical

basis, because it may be derived from the concept of electron conduction across an activation energy barrier at an interface by charge carriers having a Boltzmann distribution of energies and constant charge carrier concentration.^{3,105}

We might like to test the hypothesis of an interfacial activation energy barrier in a particulate photobiological system by testing for conformity to equation 5. Unfortunately, a *direct* test is impossible because the eye melanin particles and photosynthetic particle are so small that one cannot make electrical contacts to the two sides of the interface in order to measure the voltage-current curve. An indirect test however, is possible because equation 5 leads to a prediction regarding the shape of the decay of concentration of unpaired electrons vs time after the end of illumination, which can be measured by electron spin resonance.

We may derive the concentration vs time curve as follows:

Let us consider a liquid-solid interface at a particle surface, across which electron current flow is described by equation 5. In the solution outside the particle, let there be an ion (a small molecular weight substance) which can exist in either the oxidized or reduced state, depending on whether it has gained or lost an electron from the nearby solid surface. This reaction may be written



where x_{ox} and x_r are the oxidized and reduced forms of substrate molecule X respectively, and e^- represents an electron, which may be rapidly and reversibly exchanged between the molecule X and the solid surface of the biological particle. According to theory and experiment of electrode physics, the particle surface then experiences an electrical potential (V) given by the familiar electrode equation

$$V = V_0 + (RT/F) \log_e (x_{ox}/x_r) \quad (6)$$

where x_{ox} and x_r are concentrations of oxidized and reduced forms respectively of substrate molecule X, V_0 is a constant, and the other symbols are constants as previously defined. Because the sum of oxidized plus reduced forms of X-substrate is always constant ($= C_x$), we may write

$$x_{ox} + x_r = C_x \quad (7)$$

Combining equations 6 and 7, we obtain

$$V = V_0 - (RT/F) \log_e [x_r/(C_x - x_r)] \quad (8)$$

If as assumed, light kicks electrons across the particle surface, it disturbs the equilibrium between the two sides of the surface and sets up an electrical potential difference across the surface. Decay of photogenerated unpaired electrons then consists of conduction of electrons back across the

surface in accord with the conduction equation (equation 5), driven by the potential difference of equation 8 for the potential on the liquid side of the surface minus a constant potential which we shall assume for simplicity on the solid side of the surface (this merely assumes an electron concentration in the solid that is large compared to the number of electrons transferred). *The surface reaction described above is equivalent to an electric circuit consisting of a diode connected across a battery.*

We may then equate the driving potential across the interface (given by equation 8 minus a constant) to the voltage drop across the interface given by equation 5 to yield

$$-\frac{RT}{F} \log \left[x_r / (C_x - x_r) \right] = \frac{RT}{Fa} \log(-i) + B \quad (9)$$

where B is a new constant. Because the conduction of one mole of electrons across the particle surface reduces (or oxidized) one mole of substrate molecules, we may write

$$i = F dx_r / dt \quad (10)$$

where t is time. Substituting equation 10 in equation 9, we obtain

$$-\frac{RT}{F} \log \left[x_r / (C_x - x_r) \right] = \frac{RT}{Fa} \log \left[-F \frac{dx_r}{dt} \right] + B \quad (11)$$

which is easily converted to

$$-\frac{dx}{dt} = \frac{1}{Fg} \left[\frac{x}{C_x - x_r} \right]^a \quad (12)$$

where g is a new constant. If $a = 1$, as is theoretically the case for an ideal electrode surface,¹⁰⁸ and if reaction velocity (v) is defined as $v = dx/dt$, then equation 12 may be written in the form

$$\frac{1}{v} = \frac{Fg}{1} \left[\frac{C_x}{x_r} - 1 \right] \quad (13)$$

which may be compared with the classical rate equation of Michaelis-Menten for enzyme kinetics

$$\frac{1}{v} = \frac{1}{V} \left[\frac{K_s}{s} + 1 \right] \quad (14)$$

where s is substrate concentration and V and K_s are constants.

The rate equation of classical enzyme kinetics (equation 14) is derived from the hypothesis of reactants in free solution obeying the mass action principle, while equation 13 was derived from solid state and electrode physics concepts. Equation 13 and 14 both predict linearity of a double

reciprocal plot of reaction velocity vs substrate concentration, but differ in that they predict opposite signs for the intercepts on the $1/v$ - axis.

The relevance of the solid state activation energy barrier equation (equation 13) to biology is supported by the fact that decay of photo-generated free radicals in eye melanin particles (Fig. 2) conforms to equation 13 and so does the decay of photoconductivity (Fig. 3) in invertebrate nerve.

Equation 12 for activation barrier kinetics may be put into another form, which is sometimes more convenient for application to experimental data, and which shows an important correlation with experimental solid state physics. This is true because the function $\log [x_r/(C_x - x_r)]$ may be adequately approximated by a linear function of x_r which may be shown in the following way: Let us define a new variable λ equal to that fraction of total substrate concentration which is present in the reduced form. Hence, λ is defined by

$$\lambda = x_r/C_x \quad (15)$$

A graph of (Fig. 4) of the function $\log_{10}[\lambda/(1-\lambda)]$ vs λ shows that $\log_{10}[\lambda/(1-\lambda)]$ can be expressed fairly accurately as a linear function of λ for values of λ between approximately 0.15 and 0.85. By measuring the slope and intercept of the straight line approximation, we may write the approximate equation

$$\log_{10} \left[\frac{\lambda}{1-\lambda} \right] = 2\lambda - 1 \quad (16)$$

The right hand side of equation 16 is actually the first two terms of the Taylor series expansion of the left hand side. Substitution of equation 15 in equation 16 and conversion to $\log$ to the base e gives

$$\log_e \left[\frac{x_r}{C_x - x_r} \right] = \frac{4.6x_r}{C_x} - 2.3 \quad (17)$$

Equation 17 may be written in the form

$$\left[\frac{x_r}{C_x - x_r} \right] = \exp \left[2.3 \left(\frac{2x_r}{C_x} - 1 \right) \right] \quad (18)$$

Equation 18 may be substituted in equation 12 to give

$$-\frac{dx_r}{dt} = me^{nx_r} \quad (19)$$

where m and n are new constants defined by

$$m = \frac{A'}{Fg} \exp(2.3a) \quad (20)$$

EYE MELANIN FREE RADICALS

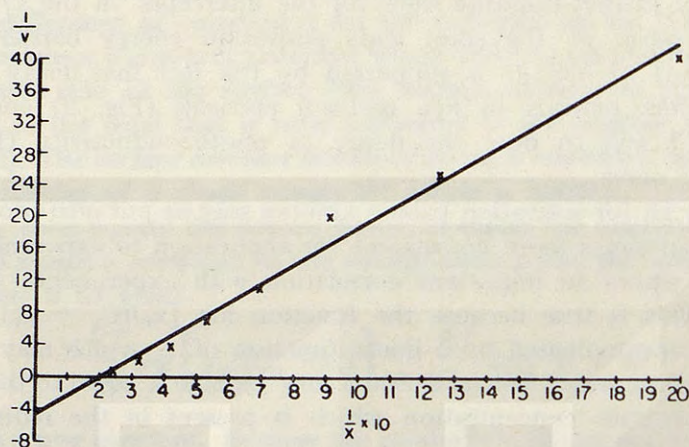


Fig. 2. Double Reciprocal Plot for Decay of Eye Melanin Free Radicals. x = substrate (free radical) concentration. v = reaction velocity = $-dx/dt$. This graph shows a *negative* intercept on the $1/v$ -axis, which may be contrasted with the *positive* intercept seen in the usual Lineweaver-Burk plot of data from an enzyme reaction conforming to Michaelis-Menten kinetics. Experimental data was obtained from electron spin resonance analysis of eye melanin particles in aqueous suspension at end of illumination with visible light.¹⁰⁹

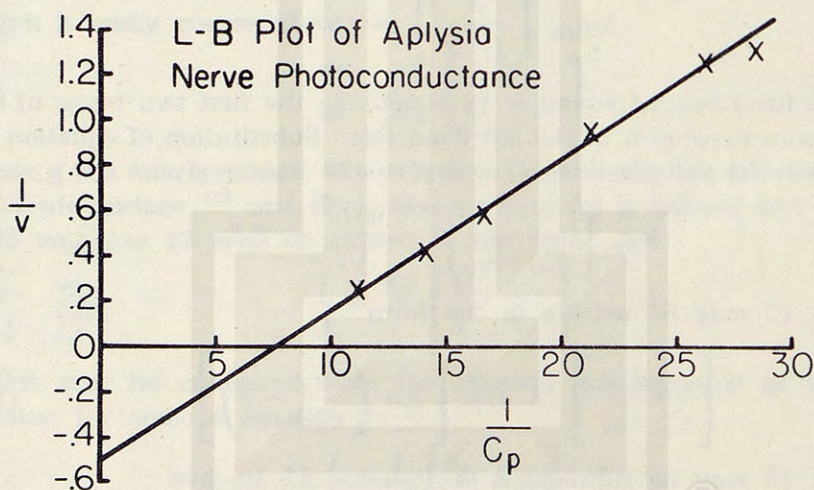


Fig. 3. Double Reciprocal Plot for Decay of Photoconductivity in Nerve. C_p = photoconductance in $(M \Omega)^{-1}$ of membrane of *Aplysia* neuron; $v = dC_p/dt$, where t is time. Experimental data were taken from figure 9 of Chalazonitis,¹¹⁸ in which the data points represent microelectrode measurements of electrical resistance across the membrane of the photo-active neuron of *Aplysia* at various times after the end of exposure to 18 seconds of light with a wavelength of 470-510 $m\mu$. Calculations are taken from Cope.¹¹⁹

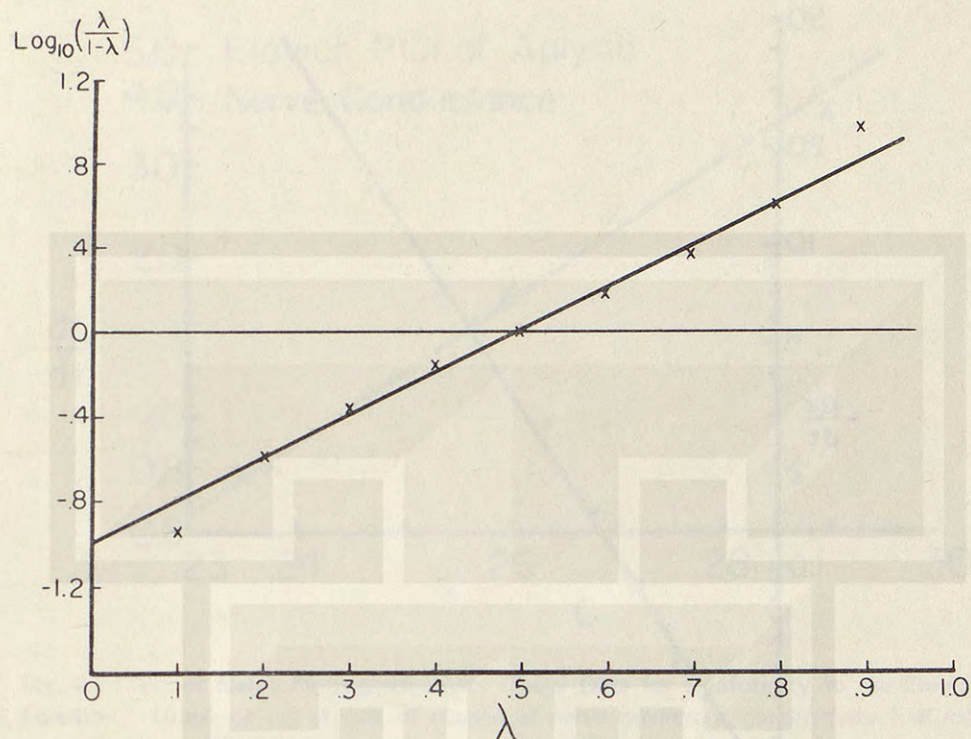


Fig. 4. Plot λ vs. the log function. The accuracy of this linear approximation is studied in the appendix of Reference 3.

and

$$n = \frac{4.6a}{C_x} \quad (21)$$

Equation 19 is called the Roginsky-Zeldovich equation or the Elovich equation.¹⁰⁹ It is named after its discoverers who observed that it described data on oxygen absorption and charge decay at surfaces of inorganic semiconductors. The applicability of the Elovich equation to experimental data of surface physics of semiconductors, such as Ge and Si, has been confirmed repeatedly.¹⁰⁹ The frequency of experimental observation of this equation suggested that it was a manifestation of fundamental physico-chemical principles of surfaces, probably connected with electron transport processes. A variety of derivations of the Elovich equation have been

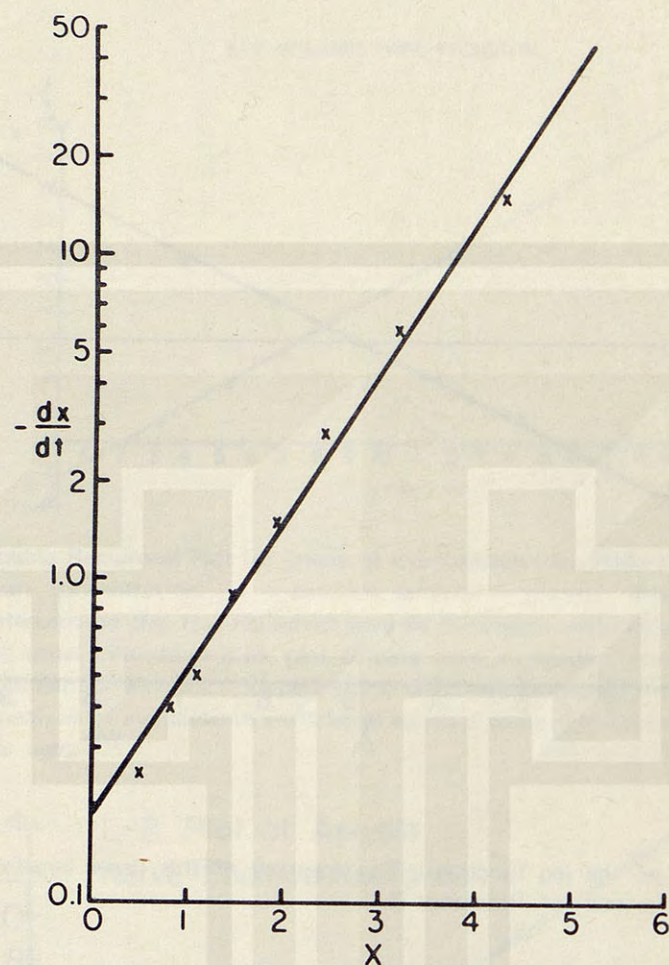


Fig. 5. Test of Eye Melanin Free Radical Decay Data for Conformity to Elovich Equation. Graph of log of reaction rate ($-dx/dt$) vs free radical concentration (x) for the experimental data of Fig. 2. Linearity of this graph indicates conformity of this data to the Elovich equation (equation 19).

proposed, but all seem to be based on postulates of doubtful validity.¹⁰⁹ The derivation presented here represents a new approach, which is based on postulates that have a good basis in experiment and theory.¹⁰⁹

After the Elovich equation had been derived by Cope¹⁰⁹ from solid state activation barrier theory, its relevance to biology was confirmed by showing that it described considerable biological data. The Elovich equation describes well the decay of free radicals in eye melanin particles (Fig. 5) and the decay of photoconductivity in nerve (Fig. 6). It also described the decay of free radicals in photosynthetic particles,¹¹⁰ and the decay of molecules responsible for delayed emission of light from green leaves.¹¹¹ All of these biological systems contain particles or membranes

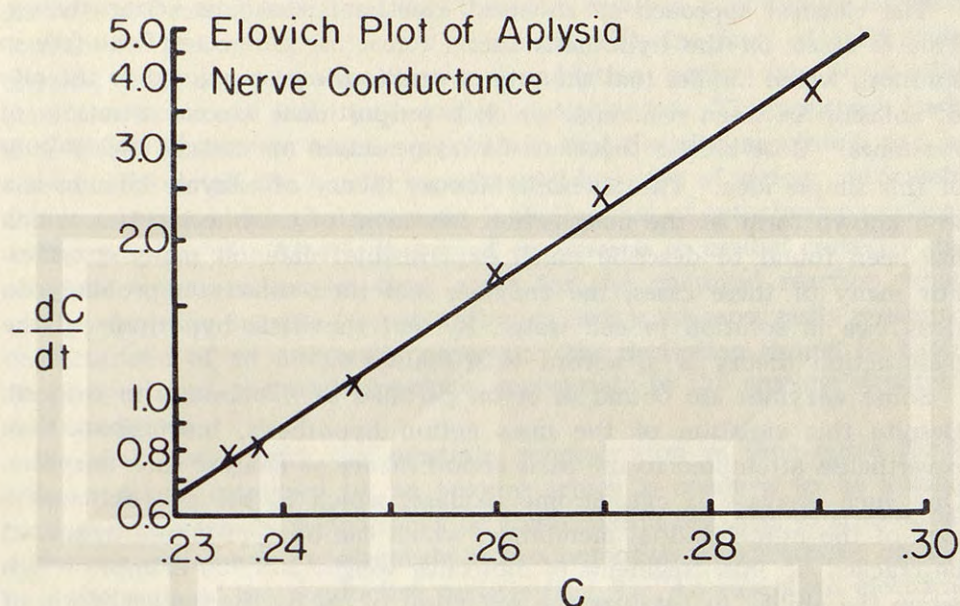


Fig. 6. Test of Nerve Photoconductivity Decay Data for Conformity to the Elovich Equation. Graph of log of rate of change of nerve membrane conductivity ($-\frac{dC}{dt}$) vs conductivity (C) for the experimental data of Fig. 3 on *Aplysia* nerve membrane, using the data of Chalazonitis¹¹⁸ as calculated by Cope.¹¹⁹ Linearity of this graph indicates conformity of this data to the Elovich equation (equation 19).

as required by the activation overvoltage derivation of the Elovich equation. Support for the concept that solid state electron conduction may be involved in the eye melanin reaction derives from the measurement of relatively high electron mobilities in eye melanin by the new experimental technique of microwave Hall effect.¹⁵

In addition, the temperature dependence of the Elovich equation has been derived from activation overvoltage theory, and has been found to agree well with experiment, both for inorganic solid systems and for biological systems.¹¹²

C. Enzymes (Cytochrome Oxidase). Enzymes are the catalysts in cells which cause the chemical reactions necessary for life to proceed at rapid rates. Without enzymes these chemical reactions would occur too slowly to support life. Because of their importance to life, the structure of enzymes, and the kinetics and mechanisms of chemical reactions catalyzed by enzymes, have been studied extensively by biochemists and physical chemists for many years.

The classical approach to chemical kinetics is the mass action theory. This is based on the hypothesis that all reacting molecules float free in solution, which implies that the rate of reaction is proportional to the rate of collision between reactants, which is proportional to concentrations of reactants. Most kinetic theories of enzyme action are merely elaborations of this simple idea. The Michaelis-Menten theory of enzyme kinetics is a well known form of the mass action treatment of enzyme kinetics, which has been found to describe much experimental data on many enzymes. For many of these cases, the enzymes and their substrates probably do float free in solution in cell water, so that the basic hypothesis of the mass action theory is in accord with reality.

Some enzymes are bound in or on particles or membranes in the cell. Despite this violation of the mass action hypothesis, biochemists have nevertheless attempted to use mass action theory to analyze such enzymes. One such enzyme is cytochrome oxidase, which in the cell exists as a part of the mitochondrial membrane, which can by appropriate treatment be cracked up into fragments of molecular weight 500,000 or more which retain the ability to catalyze the reduction of O_2 by the reduced form of the protein cytochrome c. The membrane fragment called cytochrome oxidase contains protein, unsaturated lipids, at least two types of haeme-iron groups, and copper. Purifications which remove any of these substances cause loss of catalytic activity of the enzyme.

Despite the fact that cytochrome oxidase is part of a membrane and hence violates the free solution hypothesis of mass action theory, biochemists have nevertheless tried to analyze its kinetics on the basis of mass action theory. The results have been poor. Cytochrome oxidase does *not* conform to the Michaelis-Menten pattern. Instead, it shows first order kinetics, with the peculiar feature that larger initial amounts of reduced cytochrome c substrate decrease the first order rate constant in a hyperbolic pattern. Such a result is difficult to derive from mass action theory. It can be done by assuming a four step reaction, *if the rate constants of the first and last step are assumed to be equal*.¹¹³ The latter is possible, but seems rather unlikely.

Persistence by biochemists in attempts to apply mass action theory to membrane-bound enzymes which violate its postulates is psychologically understandable because of the great success of mass action theory in dealing with soluble enzymes. Reason dictates, however, that when reality conflicts with the postulates of theory and when predictions of theory are difficult to reconcile with experiment, then a different theoretical approach should be sought. This has been done by Cope^{10,12,114} in order to deal with the kinetics of redox reactions in biological particles and membranes using realistic postulates. No longer does one impose the restrictive hypothesis of mass action theory that all processes must occur

in free solution. One concedes that the catalytic system contains solid particles and membranes, and that therefore catalysis may involve electron conduction through these solids in accord with the laws of solid state physics, and may involve electron conduction across the liquid-solid interfaces in accord with the behavior of electrodes. This approach leads in a direct manner to kinetic predictions that describe accurately and in considerable detail the kinetic experimental behavior of various particulate and membranous biological processes, including that of cytochrome oxidase.

The purpose of a kinetic theory of enzymes is to predict correctly the substrate concentration vs time curve for the chemical reaction when catalyzed by the enzyme (concentration vs time is the most easily measured characteristic of an enzymatic process). The derivation should be based on physical and chemical principles appropriate to the enzyme structure and conditions.

We shall now derive the substrate concentration vs time curve for a redox reaction catalyzed by an enzyme which is assumed to be a solid particle across which electrons can be conducted in response to a potential difference generated by electrode redox potentials of substrates on the opposite ends of the conductive particle (Fig. 7).^{*} An equivalent electrical circuit would be a resistor connected across a battery. This model seems appropriate for an enzyme such as cytochrome oxidase which when studied in the test tube is a high-molecular weight fragment of a membrane. In life, cytochrome oxidase exists as part of the membrane of the mitochondrion. When in this larger and more complex solid system, some additional solid state processes may influence the cytochrome oxidase reaction, as will be discussed later in this section. The derivation, which will now be presented in simplified form, can be found in complete detail elsewhere.^{10,12,114}

Let us postulate an enzymatic particle (or membrane fragment) as shown in Fig. 7. Let there be two sites on the particle at which small molecular weight substrate molecules can exchange electrons with the particle. The two substrates (X and Y) can each exist in an oxidized or reduced form. We assume enzymatic specificity of site for substrate: in other words, the X-site on the particle can exchange electrons only with X-substrate, and the Y-site can exchange electrons only with the Y-site.

Let us now consider the system shown in Fig. 7 from the point of view of solid state theory. The X-site and Y-site may be considered as two electrodes immersed in two solutions with different equilibrium electrode potentials. When the two electrode sites are connected by a wire, or by the internal resistance of the enzyme particle, a current of electrons will flow, which will be limited by the electrical resistance across the particle. This solid state model of an enzyme is electrically equivalent to a resistor

^{*}Digby¹⁷⁰ independently proposed that this same mechanism operates across the shells of crabs and shrimps. The same mechanism had been proposed earlier by Lund¹⁷³⁻¹⁷⁵ and by Jahn.¹⁷⁶

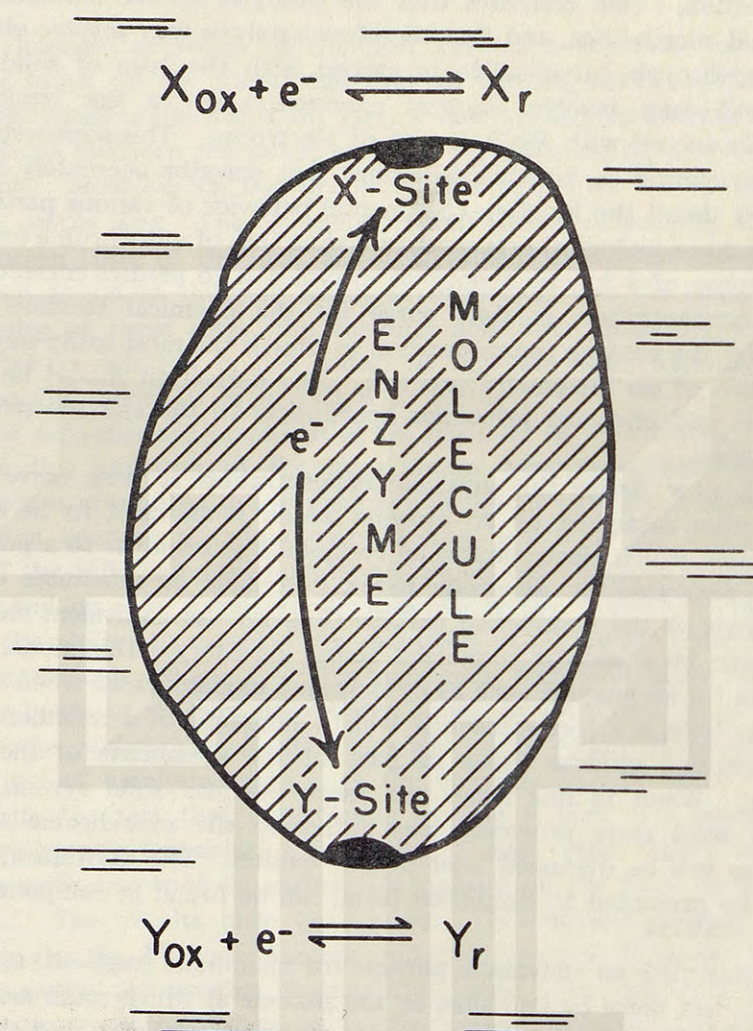


Fig. 7. Solid State and Electrode Model of the Cytochrome Oxidase Enzyme Particle. X-site on the particle acts like an electrode immersed in a solution of redox substrate X, so that the X-site develops an equilibrium electrode potential governed by the percent reduction of substrate X. The X-site is assumed to have enzymatic specificity for reaction with X-substrate. In other words, X-site can exchange electrons freely with X-substrate but will not react with Y-substrate. The Y-site, at the other end of the enzyme particle acts like a second electrode immersed in a solution of a second redox substrate Y, and develops an equilibrium electrode potential governed by percent reduction of Y. The direct reaction of X- and Y-substrates in solution is assumed to be insignificantly slow. The difference in electrode potentials at the X- and Y-sites causes a current of electrons to flow through the conductive solid enzyme particle. The rate of current flow, and hence the rate of oxidation of X-substrate by Y-substrate, is governed by the over-voltage phenomena at the X- and Y-sites, and by the electrical resistance across the solid enzyme particle.

connected across a tiny battery. To develop the theory of this behavior, we shall express the site (or electrode) potentials and the voltage-current equation in terms of substrate concentrations and time.

First, let us calculate the potential difference across the particle (Fig. 7), which we shall call the cell potential (V_{cell}). This is merely the difference in equilibrium electrode potentials experienced by the two sites at the two ends of the particle. Hence:

$$V_{cell} = V_x - V_y \quad (22)$$

where V_x and V_y are the electrode potentials at the X- and Y-sites respectively. Equation 22 may be put in terms of substrate concentration by the use of the familiar electrode equation

$$V_x = V_x^0 + (RT/F) \log_e(x_{ox}/x_r) \quad (23)$$

where x_{ox} and x_r are the concentrations of the oxidized and reduced forms of X-substrate, R is the gas constant, T is absolute temperature, F is the Faraday and V_x^0 is a constant. Because the sum of oxidized plus reduced forms of X-substrate is always constant ($= C_x$), we may write

$$x_{ox} + x_r = C_x \quad (24)$$

We shall assume for simplicity that Y-substrate is present in excess, so that the redox potential at the Y-site on the particle is constant ($= Y$). We may then combine equations 22, 23, and 24 to obtain the potential difference across the particle (V_{cell}) (Fig. 7) in terms of concentration of reduced substrate as follows:

$$V_{cell} = V_0 - (RT/F) \log \left[x_r / (C_x - x_r) \right] \quad (25)$$

where V_0 is a new constant ($= V_x^0 - Y$).

The potential difference across the enzyme particle causes a flow of electron current across the particle, like that which would occur through a resistor connected across a pair of electrodes in a battery. If we assume that current flow (i) across the enzyme particle is governed by Ohm's law, as is true for most organic semiconductors, we may write

$$V_{cell} = ir \quad (26)$$

where r is the electrical resistance across the enzyme particle.

Because the conduction of one mole of electrons across the enzyme particle reduces (or oxidizes) one mole of substrate, we may write

$$i = F dx_r/dt \quad (27)$$

We may now derive the differential equation for substrate reduction by substituting equations 25 and 27 in equation 26 to obtain an equation of the form

$$-\frac{dx_r}{dt} = A \log \left[\frac{x_r}{C_x - x_r} \right] - B \quad (28)$$

where x_r is concentration of the reduced form of X-substrate, t is time, C_x is total concentration of oxidized plus reduced forms of X-substrate, and A and B are constants.

Equation 28 is the basic differential rate equation describing the kinetics of a particulate enzyme that is rate-limited by ohmic conduction of electrons across the particle. Unfortunately, because of the log term, equation 28 cannot be integrated by any exact method. We may, however, test experimental data for ohmic conduction kinetics by plotting a graph of (dx_r/dt) vs $\log [x_r/(C_x - x_r)]$, although this is rather inconvenient. Linearity of such a plot would indicate conformity of the data to equation 28.

Fortunately, equation 28 may be converted to an approximate form of reasonable accuracy, which is easy to integrate and easy to use for the analysis of experimental data. We may substitute the approximate equation 17 in equation 28 to give a new differential rate equation for a particulate electron conduction equation having the simple form

$$-\frac{dx_r}{dt} = \left[\frac{\alpha}{C_x} \right] x_r + \delta \quad (29)$$

where α and δ are constants. This approximation¹⁰ is moderately accurate from 15 percent to 85 percent reduction of substrate (Fig. 4).

Equation 29 was derived on the assumption that it is the only voltage drop in the equivalent circuit of Fig. 7. If we make our picture more realistic by assuming that a diffusion overvoltage at the X-site also exists due to slow diffusion of large substrate molecules and that Y-substrate is also significantly reduced during the course of the reaction, then the differential equation of reaction kinetics remains of the same general form as equation 29, but with some extra constants and becomes

$$-\frac{dx_r}{dt} = \left[\frac{\alpha}{C_x + \beta} + \gamma \right] x_r + \epsilon \quad (30)$$

where β is a positive constant that is a function of the diffusion constant of substrate x_r , γ is a positive constant and ϵ is a constant. Hence, like equation 29 for the simpler model, the improved theory predicts a hyperbolic relationship between the first order rate constant (k') and C_x , but of a more complicated form as follows:

$$k' = \frac{\alpha}{C_x + \beta} + \gamma \quad (31)$$

or

$$(k' - \gamma) \cdot (C_x + \beta) = \alpha \quad (32)$$

Equations 29 and 30 predict the kinetics of substrate reduction to be expected from an enzyme particle which is rate limited by ohmic conduction of electrons across the solid particle without or with a diffusion overvoltage at the particle surface. The kinetics are predicted to be of first order with respect to the concentration of reduced substrate (x_r). The important and unusual special feature of the prediction is that the first order rate constant (k') should decrease if larger *total* (oxidized plus reduced) concentrations (C_x) of substrate are used, and the relationship is predicted to be hyperbolic of the form given by equation 32.

A kinetic pattern of exactly this form has been observed experimentally with the enzyme cytochrome oxidase when catalyzing the oxidation of reduced cytochrome c by O_2 and has also been observed for the enzymes cytochrome peroxidase¹⁰ and pyruvate carboxylase.¹¹⁴ To account for this unusual behavior in terms of classical mass action mechanisms, one would have to postulate some sort of substrate inhibition, or a complicated sequence of reactions.¹¹³

In addition to predicting the kinetic pattern of the cytochrome oxidase reaction, the solid state theory predicts the experimentally observed temperature dependence of this enzyme.¹² The theory given here predicts that the activation energy of the functioning cytochrome oxidase enzyme should equal the semiconduction activation energy for the solid protein of the enzyme particle.¹² Solid state physical measurements made recently on purified dried cytochrome oxidase do indeed agree with this prediction, and show that cytochrome oxidase is unique among proteins thus far studied, in that it has a semiconduction activation energy three times lower than other proteins.^{11,12} Additional support for the concept that cytochrome oxidase activity involves solid state electron conduction is provided by measurements of relatively high electron mobilities in mitochondria by two new experimental techniques (pulsed electron beam^{5,9} and microwave Hall effect¹³⁻²²).

When the cytochrome oxidase reaction is studied at low oxygen concentrations, the rate equation of the reaction is observed to change from first to second order, the oxygen concentration shows an inhibitory effect on the second order rate constant of hyperbolic form.¹¹⁵ These experimental findings are quantitatively understandable from the same concept of a solid state electron conduction enzyme, with the additional features of space charge injection of electrons (yielding square law instead of ohmic conduction) and trapping.¹¹⁶

According to intuition, and also according to detailed theory,¹² if cytochrome oxidase functions by a solid state mechanism, then the

activation energy of the cytochrome oxidase reaction should approximately equal the activation energy for semiconduction in dried cytochrome oxidase, as measured by solid state physical techniques. For various simple proteins, semiconduction activation energies as measured by numerous investigators had always been found to be in the region of 1.0 eV. Since the cytochrome oxidase enzymatic reaction had been observed to have an activation energy of 0.3 eV or lower, a semiconductive mechanism for enzyme action had been thought unlikely. The possibility was overlooked that cytochrome oxidase protein might have a different activation energy of semiconduction from most other proteins. When at long last it was measured,^{11,12} the semiconduction activation energy of dried cytochrome oxidase was found to be approximately 0.3 eV, which supports the solid state physical concept of the cytochrome oxidase mechanism.

Direct experimental measurements of electron conductivity under physiological conditions in cytochrome oxidase and in other systems that show solid state kinetics would be desirable to test theoretical deductions. Such measurements present great technical difficulties. The conductivity and electron mobility techniques generally used in inorganic solid state physics require samples that are single crystals yet are big enough to allow the attachment of electrodes, purity to parts per million, absence of water, and ohmic contacts between electrode and sample. Biological samples usually satisfy none of these criteria. Therefore, conventional solid state physical techniques have proved useful only to a limited extent. New techniques to avoid the problems inherent in biological samples are under development and have yielded some interesting preliminary data.

The only study by conventional solid-state techniques that has proved illuminating is the measurement by Straub^{11,12} of the activation energy of semiconduction of purified, dried cytochrome oxidase. This showed that cytochrome oxidase was unique among the proteins thus far studied in having an activation energy threefold lower than others, in agreement with the predictions of solid state kinetic theory. With regard to new techniques, preliminary results are available from a pulsed electron beam method developed in Russia,^{5,9} which showed relatively high electron mobilities in mitochondria. This result, which agrees with theoretical expectations, has been confirmed in preliminary measurements with a new microwave Hall-effect technique by Eley and Pethig.¹⁸ The method was invented in Russia by Trukhan^{13,14} and improved in England by Eley and Pethig.^{16,22} More recently, Eley *et al*^{19,20} showed that cytochrome oxidase activity is positively correlated with microwave Hall mobility of electrons, which strongly supports the concept that cytochrome oxidase enzyme activity is rate-limited by solid state electron conduction.

In conclusion, the evidence in support of a solid state mechanism for cytochrome oxidase now seems fairly strong.

The theory just presented describes well the experimental behavior of cytochrome oxidase in reactions in the test tube. In life, however, cytochrome oxidase exists as a part of a much larger solid membrane system, which performs at least one important biochemical function in addition to what is done by purified cytochrome oxidase in the test tube. In life, cytochrome oxidase exists as part of the membrane of mitochondria (small granules present in every cell which are barely visible under the light microscope). Cytochrome oxidase participates in the oxidative or electron transport process of the mitochondria. In mitochondria which are undamaged, the energy from oxidation is converted into high energy phosphate bond energy by phosphorylation of adenosine diphosphate (ADP) to adenosine triphosphate (ATP) which is the immediate source of energy for most body functions (muscle contraction, nerve firing, secretion of acid in the stomach, etc.).

The mechanism of coupling between oxidation and phosphorylation in mitochondria is not understood by biochemists. Various biochemists present various explanations with an air of confidence, but the various explanations are different, so that at least some of them must be wrong.

If one concedes that the *oxidative* part of the oxidative phosphorylation of cytochrome oxidase might occur in a solid, then one may ask whether *phosphorylation* and the coupling process might also occur by solid state mechanisms. Preliminary theoretical considerations suggest that this is indeed possible. No experimental tests of this hypothesis have yet been made.

It was suggested by Straub^{11,23,24} that phonons (packets of lattice vibrational energy) might be the immediate source of energy for the phosphorylation in the mitochondrial solid. It was then suggested by Cope and Straub¹² that the coupling process was a polaron (a combination of a phonon and an electron), which could be conducted across the mitochondrial solid and would give up its phonon for a phosphorylation. The evidence that suggested polaron conduction was derived from an analysis in terms of solid state kinetic theory of the temperature dependence of the cytochrome oxidase reaction as measured by deVault and Chance.²⁷

If the carrier of the energy for phosphorylation is the phonon, we may inquire about the mobility of this carrier for transport of the energy through the mitochondrial solid. We must first note that the free energy of hydrolysis of adenosine triphosphate is 9.0 kcal/M or 0.39 eV, which must be the energy of phosphorylation of ADP. Hence, the phosphorylation phonon must carry at least this much energy, which is equivalent to a photon of light of 3.24μ wavelength, which is in the infrared (IR) range. Phonons of this energy range are localized vibrations (optical phonons) and do not significantly propagate through the solid, in contrast to lower energy

phonons (acoustic phonons) which move freely. It therefore seems unlikely that phonon movement can play a significant role in dissemination of energy through the lattice of the solid mitochondrial membrane. If the solid state carrier of energy should be an exciton, this might be either free or localized.²⁵

A different method of dissemination of an energy packet of this size is suggested by the dimensions of the mitochondrion (3.3μ long by 0.5μ radius for rat liver). Because the length of the mitochondrion is approximately one wavelength of this phonon energy packet, Cope²⁵ considered the possibility that the mitochondrion might act as a resonant cavity for IR electromagnetic waves, which could serve as a rapid and efficient method for dissemination of IR energy throughout the mitochondrion from generation sites to utilization sites. The mitochondrion would then be a small scale analog of a microwave cavity made from a shorted section of waveguide, as used in radar systems.

A serious objection to this idea is the fact that water, which is the principle constituent of the mitochondrial sap, absorbs IR energy strongly, so that IR radiation within the mitochondrion would be mostly absorbed by water and wasted, instead of being transmitted.

A variation of the above mechanism would allow the mitochondrion to avoid the IR absorption by water.²⁵ Suppose that the IR radiation does not pass into the aqueous sap but is contained within the lipid bilayer of the mitochondrial membrane. Suppose that IR electromagnetic waves are generated at the surface of the lipid bilayer and kept within the lipid bilayer by internal reflections from the adjacent layers of protein. The mitochondrion would then be analogous to a coaxial transmission line where the lipid bilayer is the dielectric and the adjacent protein layers are analogous to the metal surfaces of the central wire and of the outer metal sheath.²⁵ This coaxial transmission line, when shorted at both ends, forms a coaxial cavity which is resonant for any integral multiple of a half wavelength (the multiple is 2 for the mitochondrion for 3.24μ wavelength energy packet). The composition and geometrical structure of the mitochondrial lipid bilayer minimize absorption of IR waves by the lipid dielectric, so that this could be a highly efficient method for energy propagation throughout the mitochondrial membrane.

The first reason for expecting low absorption, and hence low wastage of IR energy within the lipid bilayer of the mitochondrial membrane results from its chemical composition.²⁵ Analysis of mitochondrial lipids reveals that they consist mostly of compounds with very low IR absorption, including 33 percent saturated straight-chains (16-18 carbons), and 52 percent straight chain (18-20 carbons) with 2 or 3 double bonds. Indeed, the IR absorption of saturated straight-chain hydrocarbons is so low that such compounds (Nujol) are commonly used to make slurries of crystals for IR spectroscopy. The only significant IR absorption in saturated

straight chains is the C—H stretching frequency, which is located near 3.3μ . In unsaturated carbon chains, there is also an IR absorption band due to the C=C stretching frequency, which is very weak, although it becomes more intense when conjugation occurs with multiple double bonds.

Even though IR absorption in the mitochondrial lipid bilayer must already be low because of the chemical composition of the lipid, it must be reduced lower by the particular geometrical arrangement of lipid molecules relative to the geometrical pattern of the electric vector, which is produced in a coaxial cavity of this type.²⁵

The electric field configuration to be expected in the IR coaxial cavity consisting of the mitochondrial membrane may be deduced easily from the theory of coaxial transmission lines. For a coaxial transmission line, at a wavelength (λ) longer than the cutoff wavelength (λ_c), only the principle transverse electromagnetic (TEM) mode can be propagated, where λ_c is given approximately by

$$\lambda_c = \sqrt{\epsilon} \pi (r_1 + r_2) \quad (33)$$

where r_1 and r_2 are the inner and outer radii of the coaxial transmission line, and ϵ is the dielectric constant.²⁵ For the rat liver mitochondrion, radius $\cong 0.5\mu$ and the thickness of the lipid bilayer dielectric is relatively small ($60 \text{ \AA} = 6 \times 10^{-3} \mu$), so that $r_1 \cong 0.5\mu$. If we take the dielectric constant (ϵ) of the lipid bilayer as one, then we may substitute these numbers in equation 33 to obtain the cutoff wavelength for the mitochondrion $\lambda_c = 3.0\mu$. Because the wavelength of the energy packet for phosphorylation is 3.24μ which is longer than λ_c , only the principle TEM mode can be propagated in the mitochondrial membrane.²⁵ Higher order modes with other field configurations cannot exist.

The field configuration for the principle TEM mode in a coaxial transmission line has the electric vector (E) directed radially and the magnetic vector (H) directed circumferentially, which must therefore be the situation in the mitochondrial membrane. According to the Danielli model of the lipid bilayer membrane, the carbon chains of the lipid molecules are directed perpendicular to the membrane surface, so that in the mitochondrial membrane, the carbon chains of the lipids are directed radially, which is parallel to the direction of the E vector. The C—H bonds are perpendicular to the chains and are therefore perpendicular to the E vector. As mentioned before, the principle IR absorption of straight carbon chain hydrocarbon molecules is the C—H stretching frequency. It has been shown by both theory and experiment that IR absorption by a molecular group is a minimum (which may be zero) when the E vector is perpendicular to the direction of the molecular vibration. It is therefore to be expected that the absorption of IR energy by the lipid bilayer of the mitochondrial membrane which already must be low because of the chemical composition, must be even lower because the C—H bonds are perpendicular to E. This expected

low IR absorption implies that dissemination of IR phosphorylating photons by the coaxial resonant cavity mechanism may be extremely efficient, because of very low losses expected from absorption in the lipid dielectric.²⁵

In summary, it seems likely that the mitochondrial enzyme system, which operates in an organized solid membrane, functions by an integrated set of solid state processes. Oxidation by cytochrome oxidase occurs by solid state conduction of polarons, which yield phonons which perform phosphorylation of ADP to ATP. The phonon energy is disseminated rapidly and with low loss throughout the entire mitochondria membrane by infrared electromagnetic waves in the lipid bilayer of the mitochondrial membrane, so that the mitochondrion as a whole acts as an infrared resonant coaxial cavity.

D. Nerve. Nerve fibers consist of a solid matrix of protein and lipid with interstitial water containing various small ions, such as sodium (Na), potassium (K), and chloride (Cl). When a nerve fires, a complicated set of movements of these ions occurs through the water in the nerve. Extensive measurements of these ion movements have been made, and much of the experimental data has been put together (i.e. the experimental data has been curve fitted) by a complicated set of equations called the Hodgkin-Huxley theory.¹¹⁷

The Hodgkin-Huxley theory is not an explanation of nerve conduction in terms of the principles of physical chemistry. Rather, it is merely an ingenious curve fit of much experimental data on ion flows in nerve water. It ignores completely the possibility of solid state events within the solid protein-lipid matrix of the nerve.

Evidence is now available for several different solid state physical processes in the solid matrix of nerve. These include electron conduction over activation energy barriers, superconduction, piezoelectric phenomena, and transmission of infrared electromagnetic waves in the lipid bilayer. It seems reasonable to suppose that these solid state processes take part in transmission of the nervous impulse down the nerve fiber, and are integrated with the ionic events which take place in the nerve water.

Electron conduction across activation energy barriers in nerve is evidenced by the experimental observation of Chalazonitis¹¹⁸ as analyzed by Cope¹¹⁹ that decay of photoconductivity in pigmented invertebrate nerve conforms to the Elovich equation, the derivation and significance of which was discussed in Section IIIB of this review.

A role for superconduction in nerve function is suggested by several types of evidence. These include (a) the temperature dependence of certain neural processes is characteristic of single electron superconductive tunneling,⁵⁶ (b) certain other neural processes show *negative* temperature dependences, which are difficult to explain except from the hypothesis of two electron superconductive tunneling,⁵⁶ (c) numerous behavioral effects

on organisms are caused by magnetic fields smaller than 2 Gauss, for which no physical mechanism of adequate sensitivity is known, except the superconductive Josephson effect,^{59,60} (d) dilute superconductivity has been observed experimentally⁴⁹⁻⁵¹ in various bile acids at temperatures as high as 277°K, and extrapolation of this data leads to the prediction⁵² the cholesterol should superconduct at physiological temperatures and above. Cholesterol is a major constituent of the solid matrix of nerve. In addition, solid state magnetic phenomena suggestive of superconduction have been observed experimentally in the presence of high electric fields.⁵³ Nerve possesses thin lipid layers (containing cholesterol) in which high electric fields exist. The superconductivity predicted for cholesterol at zero electric field might be enhanced by these high fields,⁵³ so that superconduction could become of significant magnitude and play a role in nerve function.

Piezoelectricity and pyroelectricity in nerve are suggested by the following evidence. The temperature dependence of the resting membrane potential of an invertebrate nerve cell shows a temperature dependence for which log of the activation energy is a linear function of temperature, which conforms to theory of piezoelectric resonance.⁹² A square pulse of increased temperature often produces in nerve systems two response transients of opposite sign at the start and end of the temperature pulse (Fig. 1). This is characteristic of a pyroelectric process.¹⁰⁰ It is seen in the fish thermoreceptor, which also shows proportionality between the time derivative of response and temperature, which is predicted from pyroelectric theory.¹⁰⁰ The muscle stretch receptor shows linearity of neural response with the logarithm of applied mechanical force. This is predicted from the concept that the neural response is governed by a piezoelectric effect in an elastic polymer macromolecule.¹⁰⁰ In addition, nerve is observed to be pyroelectric.¹⁵⁶

Infrared (IR) transmission of electromagnetic energy by a coaxial transmission line mechanism in nerve fibers (axons) seems possible.²⁵ Like in mitochondria, the probable immobility of IR phonons will limit the transfer of energy along the axon, where high mobility would be useful to move energy along the great distances of axon length. Like in mitochondria, the cylindrical lipid bilayer of the axon membrane seems well constructed to serve as a coaxial transmission line for rapid and efficient transfer of IR electromagnetic waves along the axon within the lipid dielectric.²⁵ A bundle of axons in the nerve could then be regarded as a coaxial IR fiber optic system. Supporting this concept is experimental observation of emission of IR radiation from nerve during excitation.¹²⁰

Some nerve fibers are larger in diameter than mitochondria, and consequently will have longer cutoff wavelengths and lower impedances than mitochondria. For an A-nerve fiber, such as commonly found in a mammalian motor nerve, a radius of 10 μ might be typical, from which

one may calculate using equation 33 that the cutoff wavelength (λ_c) will be 60μ . Hence, in a typical motor nerve axon for wavelengths over most of the IR range, higher order modes will carry a significant fraction of the total IR energy, so that longitudinal transmission of IR energy will be less efficient than in mitochondria or in smaller nerve fibers, and dielectric losses may be somewhat higher because E is not always perpendicular to the C-H bonds of the lipid chains. In a C-nerve fiber, such as one might find in the sympathetic system, a radius of 0.25μ might be found, which substituted in equation 33 leads to a cutoff wavelength (λ_c) of 1.5μ , which makes for efficient longitudinal IR transmission and low dielectric losses.²⁵

Impedance of a coaxial transmission line is given by (if resistive losses are small)²⁵

$$Z = 138 \sqrt{\mu_r/\epsilon} \log_{10} (r_2/r_1) \quad (34)$$

where Z is impedance in ohms, r_1 and r_2 are inner and outer radii of the coaxial line, μ_r is permeability ($= 1$ except for ferroelectrics), and ϵ is dielectric constant of the lipid bilayer. The impedance (Z) for IR transmission will be different for different nerve fibers because of differences in radius, although the thickness of the dielectric lipid layer is probably much the same in all nerves and in mitochondria, and will here be considered a constant ($= 0.006\mu$) as for mitochondria.²⁵ For a C-nerve fiber, with an inner radius (r_1) of 0.25μ , using equation 34, we may calculate impedance (Z) of 1.4Ω . However, for a A-nerve fiber with a radius of 10μ , we calculate²⁵ impedance (Z) of 0.036Ω .

Because for different nerve fibers, radius varies markedly but thickness of the dielectric lipid bilayer remains approximately constant, it is instructive to show the simple approximate relationship between the radius of a nerve and its impedance for transmission of IR waves.²⁵ We use the approximate equation (derived by Clark¹²¹ and Cope¹²²)

$$\log_e \left[\frac{\chi + \Delta}{\chi} \right] \cong \left[\frac{\Delta}{\chi + \Delta} \right] \cong \left[\frac{\Delta}{\chi} \right] \quad (35)$$

where $\chi > 0$ and $\Delta \ll \chi$. If we let $\chi = r$ (inner radius) $\cong r$ (mean radius of nerve fiber) and $\Delta =$ thickness of dielectric lipid bilayer, using 0.434 for the ratio of $\log_{10} x$ over $\log_e x$, we may substitute equation 35 in equation 34 and obtain

$$Z \cong 60 \sqrt{\mu_r/\epsilon} \left[\frac{\Delta}{r} \right] \quad (36)$$

where Z is axon impedance for IR transmission in ohms and the other symbols are as previously defined. This shows that, approximately, the axon impedance is inversely proportional to its radius.

The impedances for coaxial IR transmission for nerve fibers (1.4Ω for C-fibers and 0.036Ω for A-fibers) are much lower than the 75Ω which is usual in man-made coaxial transmission lines for radio frequency waves. Most efficient energy transfer between generator, line and load occurs when impedance of the line equals that of generator and load. One may speculate that therefore the generators and absorbing systems for IR energy in nerve should have low load impedances to match the nerve transmission line, and might be of fundamentally different character than the relatively high impedance devices ordinarily used in man-made systems. Pertinent to the possible nature of the low impedance nerve generator and loads is the evidence for superconductive phenomena in nerve at physiological temperatures as discussed in previous paragraphs of this section. Superconductive junctions (Josephson junctions) have been fabricated which will generate and detect electromagnetic energy in the microwave and IR range of wavelengths.^{123,124} These superconductive generating and absorbing systems have been observed^{32,125} to have impedances in the range of 1Ω . The expected low impedance of a superconductive IR generator or load in nerve might therefore be a reasonably good match to the calculated low impedance of an IR coaxial nerve transmission line.

Larger nerve fibers have lower impedances, with impedances inversely proportional to radius (see equation 36). Therefore, for efficient IR energy transfer, impedances of generators and loads should be correspondingly lower in larger nerve fibers. One might speculate²⁵ that if the generators and loads were uniformly distributed as sites over the circumferential area of the nerve with the same density in all nerve fibers, the number of sites per unit length of nerve would be proportional to radius, and the reciprocal of the total impedance of the unit length would be proportional to the sum of reciprocals of impedances of individual sites, whose number is proportional to circumferential area. Therefore, total impedance of generator or load sites per unit length of nerve would be inversely proportional to nerve radius, so that an equally good match of generator or load to transmission impedance could be obtained for nerves of all diameters using the same type and density of generator and load sites for all nerves. The hypothesis just expressed that sites of IR generation and absorption might be continuously distributed along the surface of the nerve lipid bilayer implies the possibility of repeated absorption and re-emission of the energy by the conductive walls (protein) as the IR energy is transmitted along the length of the nerve fiber within the dielectric lipid bilayer.²⁵

IV. Application of Solid State Physical Concepts to Ionic Phenomena in Cell Water

We may consider the cell to resemble a sponge, consisting of a solid skeleton of membranes and particles constructed of lipids and proteins, in

which electron currents may flow, governed by the laws of solid state physics.⁹² The interstices of the sponge are filled with water, in which reside dissolved proteins and small ions. NMR evidence¹²⁶⁻¹²⁸ indicates clearly that cell water possesses more structure than liquid water, and that much of the Na^+ and K^+ in the cell is not free in aqueous solution, but is associated with charged sites on macromolecules.¹²⁹⁻¹³³ The quantity of Na^+ and K^+ dissolved in tissue water is low, because of the structuring of the water. One may then make an analogy between the aqueous portion of the cell and a solid semiconductor. One may regard complexed Na^+ and K^+ as analogous to valence band electrons in a semiconductor, and free cations as analogous to conduction band electrons.^{134,135} The complexed ions exchange rapidly with, but are only slightly soluble in, the structured water. Conduction, diffusion, and potential equations for ionic processes in cell water therefore resemble those for electronic conduction in a semiconductor solid. Experimentally useful predictions regarding ion transport have been obtained from this approach.^{134,135}

An ionic form of solid state activation energy barrier kinetics has been developed by Cope^{135,135} which predicts that Na^+ and K^+ leakage from cells should obey the Elovich equation, which agrees with experimental observation. This theory requires that there be an activation barrier to ion conduction at the cell surface. The cell surface must resemble a liquid-solid interface like an electrode surface. Since the extracellular space is presumably liquid, the intracellular phase must have solid-like properties if the theory is correct. Specifically, it is necessary to assume that cell water has more structure than liquid water and consequently a low solubility for Na^+ and K^+ , and that Na^+ and K^+ exist in cells mostly associated with macromolecules rather than in free solutions. During the last few years, excellent evidence for cell water structuring from nuclear magnetic resonance (NMR)¹²⁶⁻¹²⁸ and from water absorption studies^{136,137} has become available and this has become the basis for an NMR method of cancer diagnosis.^{138,139} It has been shown that the metabolism of the cell produces much less energy than is required to operate the Na^+ and K^+ pumps that are required to maintain ionic concentrations in the cell for the liquid state model of the cell.¹⁴⁰⁻¹⁴³ Therefore, the liquid-state-free-cation model of the cell is thermodynamically impossible, but the solid state, or ion exchange resin, or structured water and associated cation model of the cell is compatible with all the evidence and with thermodynamics. An entire issue of the *Annals of the N.Y. Academy of Sciences*¹⁴⁴ was recently devoted to this problem.

Therefore, the weight of the experimental evidence supports strongly the analogy of the cell surface to a liquid-solid interface. Therefore, the application of solid state physical theory to the kinetics of ion transport

across the cell surface seems appropriate. Cope's solid state theory of cell water and ions is probably equivalent to a simplified form of the association-induction hypothesis of Ling^{141,145} and the ion exchange theory of Damadian.^{146,147}

V. The Future

New and better experimental techniques are needed for solid state biology. Biological processes function only when the system is complex, impure, noncrystalline and wet. The conventional experimental techniques of solid state physics are not usable under these conditions.

New experimental techniques suitable for solid state biology are beginning to be developed. Measurements of electron mobilities within wet protein particles by Hall effect at microwave frequencies have begun.^{13-22,148} A pulsed electron beam method for measurement of electron mobilities in wet proteins has been developed in Russia.^{5,9} Methods for measurement of dilute superconductivity, applicable to organic solids^{49-51,177} and perhaps to biological systems have been developed.

When the experimental techniques of solid state physical biology become as well developed as those of liquid state biology, the author expects that solid state processes will be found to be equally as important for biological functions as liquid state processes.

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Orthomolecular Psychiatry

Varying the concentrations of substances normally present in the human body may control mental disease.

Linus Pauling

The methods principally used now for treating patients with mental disease are psychotherapy (psychoanalysis and related efforts to provide insight and to decrease environmental stress), chemotherapy (mainly with the use of powerful synthetic drugs, such as chlorpromazine, or powerful natural products from plants, such as reserpine), and convulsive or shock therapy (electroconvulsive therapy, insulin coma therapy, pentyl-enetetrazol shock therapy). I have reached the conclusion, through arguments summarized in the following paragraphs, that another general method of treatment, which may be called orthomolecular therapy, may be found to be of great value, and may turn out to be the best method of treatment for many patients.

Orthomolecular psychiatric therapy is the treatment of mental disease by the provision of the optimum molecular environment for the mind, especially the optimum concentrations of substances normally present in the human body (1). An example is the treatment of phenylketonuric children by use of a diet containing a smaller than normal amount of the amino acid phenylalanine. Phenylketonuria (2) results from a genetic defect that leads to a decreased amount or effectiveness of the enzyme catalyzing the oxidation of phenylalanine to tyrosine. The patients on a normal diet have in their tissues abnormally high concentrations of phenylalanine and some of its reaction products, which, possibly in conjunction with the decreased concentration of tyrosine, cause the mental and physical manifestations of the disease (mental deficiency, severe eczema, and others). A decrease in the amount of phenylalanine ingested results in an approximation to the normal or optimum concentrations and to the

alleviation of the manifestations of the disease, both mental and physical.

The functioning of the brain is dependent on its composition and structure; that is, on the molecular environment of the mind. The presence in the brain of molecules of *N,N*-diethyl- α -lysergamide, mescaline, or some other schizophrenogenic substance is associated with profound psychic effects (3). Cherkin has recently pointed out (4) that in 1799 Humphry Davy described similar subjective reactions to the inhalation of nitrous oxide. The phenomenon of general anesthesia also illustrates the dependence of the mind (consciousness, ephemeral memory) on its molecular environment (5).

The proper functioning of the mind is known to require the presence in the brain of molecules of many different substances. For example, mental disease, usually associated with physical disease, results from a low concentration in the brain of any one of the following vitamins: thiamine (B_1), nicotinic acid or nicotinamide (B_3), pyridoxine (B_6), cyanocobalamin (B_{12}), biotin (H), ascorbic acid (C), and folic acid. There is evidence that mental function and behavior are also affected by changes in the concentration in the brain of any of a number of other substances that are normally present, such as *L*(+)-glutamic acid, uric acid, and γ -aminobutyric acid (6).

Optimum Molecular Concentrations

Several arguments may be advanced in support of the thesis that the optimum molecular concentrations of substances normally present in the body may be different from the concentrations provided by the diet and the gene-

controlled synthetic mechanisms, and, for essential nutrilites (vitamins, essential amino acids, essential fatty acids) different from the minimum daily amounts required for life or the "recommended" (average) daily amounts suggested for good health. Some of these arguments are presented in the following paragraphs.

Evolution and Natural Selection

The process of evolution does not necessarily result in the normal provision of optimum molecular concentrations. Let us use ascorbic acid as an example. Of the mammals that have been studied in this respect, the only species that have lost the power to synthesize ascorbic acid and that accordingly require it in the diet are man, other Primates (rhesus monkey, Formosan long-tail monkey, and ring-tail or brown capuchin monkey), the guinea pig, and an Indian fruit-eating bat (*Pteropus medius*) (7). Presumably the loss of the gene or genes controlling the synthesis of the enzyme or enzymes involved in the conversion of glucose to ascorbic acid occurred some 20 million years ago in the common ancestor of man and other Primates, and occurred independently for the guinea pig and for one species of bat and one bird, in each case in an environment such that ascorbic acid was provided by the food. For a mutation rate of 1/20,000 per gene generation and for even a very small advantage for the mutant (0.01 percent more progeny) the mutant would replace the earlier genotype within about 1 million years. The advantage to the mutant of being rid of the ascorbic-acid-synthesis machinery (decrease in cell size and energy requirement, liberation of machinery for other purposes) might well be large, perhaps as much as 1 percent; a disadvantage nearly as large (less by 0.01 percent) resulting from a less than optimum supply of dietary ascorbic acid would not prevent the replacement of the earlier species by the mutant. Hence, even if the amount of the vitamin provided by the diet available at the time of the mutation were less than the optimum amount, the mutant might still be able to replace its predecessor. Moreover, it is possible that the environment has changed during the last 20 million years

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