

BIOCHEMICAL RESPONSES TO A 28-DAY INTERVAL
BETWEEN EXPOSURES TO AIR AT 6.7 ATA

by

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SUMMARY PAGE

THE PROBLEM

To evaluate the effect of repetitive diving and the long term effect of compression/decompression on the distribution or loss of minerals, electrolytes, and protein metabolites in men subjected to standard Navy diving procedures.

FINDINGS

No consistent carry-over effect was observed following a 28-day inter-dive interval for dives to 6.7 ATA using air. Reduction in urine output with concomitant decreases in solute excretion during the first 24 post-dive hours was obviated by a deliberate increase in fluid intake. Ketosteroid excretion decreased somewhat following the first dive series and significantly so for 10 days post exposure after the second series 28 days later, a fact which suggests an extended adrenal response to diving stresses.

Serum electrolyte and mineral parameters showed no consistent pattern in either intra- or inter-dive variability.

APPLICATION

Since alterations in biochemical responses have been observed for several days following exposure to hyperbaric environments, it appears that caution should be exercised with respect to frequent repetition to pressure exposures. Additional work is obligatory to ascertain the extent and significance of the biochemical phenomena discussed.

ADMINISTRATIVE INFORMATION

This investigation was conducted as a part of Bureau of Medicine and Surgery Research Work Unit MF51.524.014-9016 - Mineral Metabolism in Submarine and Diving Environments. The present report is number 3 on that work unit. The manuscript was submitted for review on 22 October 1974 approved for publication on 27 November 1974 and designated as Naval Submarine Medical Research Laboratory Report Number 796.

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ABSTRACT

This study concerns an evaluation of the possible effects of repetitive diving and long-term effects of compression/decompression on distribution of, or loss of minerals, electrolytes, and protein metabolites in men subjected to standard Navy diving procedures.

Urinary minerals, electrolytes, nitrogen metabolites and steroids, as well as serum minerals and electrolytes, were measured in Navy divers following short exposures to air at 6.7 ATA. A comparison was made between two similar dives performed 28 days apart.

Increased fluid intake apparently prevented the reduction in urinary output, with its concomitant decreases in mineral and nitrogen metabolite excretion, that had been previously observed during the first post-dive day. Urinary ketosteroid excretion was slightly reduced after the first dive and decreased significantly for up to 10 days following the second hyperbaric exposure. This observation suggests prolonged adrenal responses to the stresses of compression/decompression.

Although serum ionized and total calcium, and magnesium levels showed considerable variability in this diving protocol, no consistent pattern of intra- or inter-dive variability was observed. These and other serum parameters measured indicate little or no carryover from the first to the second dive series performed 28 days later.

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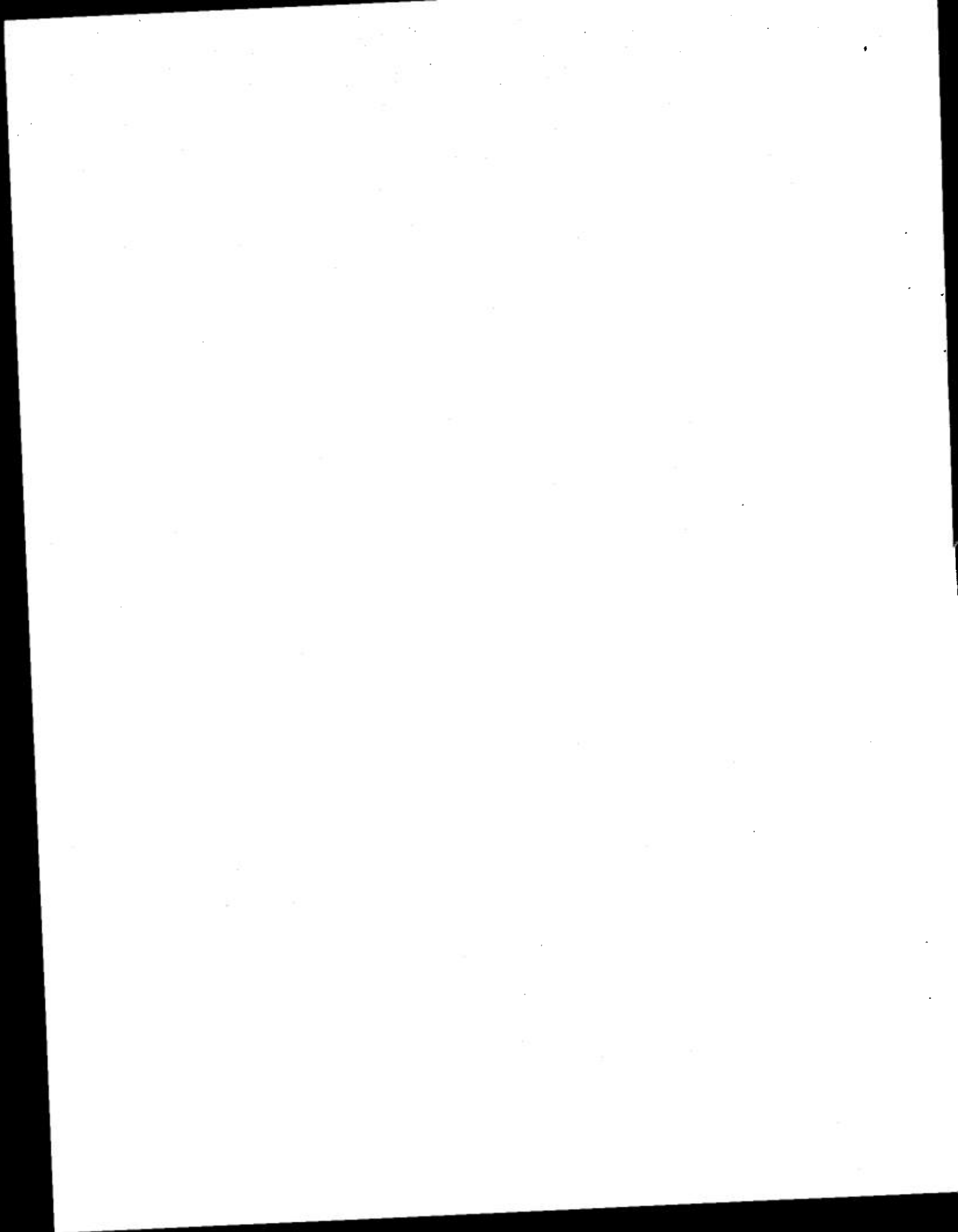
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BIOCHEMICAL RESPONSES TO A 28-DAY INTERVAL BETWEEN EXPOSURES TO AIR AT 6.7 ATA

INTRODUCTION

Alterations in blood and urinary minerals and electrolytes have regularly been observed during exposure to pressurization with subsequent decompressions,^{1,15,16} It has been suggested that these changes may be attributed to hyperventilation and fluid shifts¹⁷, increases in corticosteroid and/or aldosterone secretion³, or alterations in membrane permeability due to inert gas narcosis².

Rats exposed to severe decompression stress have been shown to exhibit serum and urinary mineral and electrolyte changes for up to 5 days post-decompression⁸. Of greater importance to the operational diver are the findings of prolonged biochemical responses following apparently non-injurious dives and decompression. Urinary mineral, electrolyte, and nitrogen metabolites were altered for up to 4 days following exposure to air at 2 and 7 ATA for 45 minutes⁹, and for up to 7 days following a simulated dive in air to 100 FSW for 60 minutes¹⁸.

It is known that exposures to hyperbaric environments result in biochemical alterations of several days duration, and certain potential effects of diving appear cumulative^{4,11} and may be related to the frequency and type of pressure experience. Therefore, the relationship between interdive interval and the accumulative affect of re-

peated pressure insults was considered appropriate for investigation.

MATERIALS AND METHODS

Four Navy divers participated in these studies. The simulated dives commenced at 0830, Tuesday through Friday, exposing one diver per day to 6.7 ATA (188 feet sea water equivalent). Compression occurred at the rate of 75 ft/min. Time on the bottom was 40-45 minutes, and decompression was performed according to the Navy Standard Diving Air Tables (190 feet for 50 min.)²⁰. Twenty-eight days after the dive the above procedure was repeated using the same subjects.

Prior to his dive each man collected five consecutive 24-hour acidified urine specimens to serve as controls. Since no individual voidings were collected during the dives, the urine samples for the first post-dive days also contained the urine produced during the dive period.

Twenty-four hour urine collections were made for 10 post-dive days. During these studies the men refrained from eating ice-cream, gelatin or soft candy since these materials contain large amounts of hydroxyproline. The method of Hosley et al¹⁰ was used to determine urinary hydroxyproline excretion.

Blood samples were drawn 3 days, 1 day and immediately prior to the dives with the average values serving as the control for each man. Blood was again withdrawn 1 hour post-surfacing and

1, 3, 5, and 7 days following the dives.

Serum to be analyzed for ionized calcium was maintained anaerobically and the level of ionized calcium was determined using an Orion specific ion flow-through electrode Model 99-20.

Total serum and urinary calcium and magnesium were measured in a Perkin-Elmer Model 306 atomic absorption spectrophotometer. Inorganic phosphorus was determined by a Technicon Autoanalyzer using a modification of method N-82 I/II while urea nitrogen, uric acid, and creatinine measurements were made using techniques N-13b and N-38a. Sodium and potassium were measured in an Instrumentation Laboratory Model 343 flame photometer; a Fiske Osmometer Model G62 was used to determine osmolality.

Urine samples were analyzed for 17-ketosteroids by the automated procedure of Zak et al.²¹ following preliminary hydrolysis, extraction with methylene chloride, two washings of the extracts with 10% NaOH, evaporation, and re-solubilization with the methanol. The same color development technique was used for hydroxysteroid analysis following treatment of the urine samples according to the procedure of Few⁵ as modified by Metcalf¹⁴.

Data analyses and statistical computations were performed using a Data General Nova 1220 computer.

RESULTS AND DISCUSSION

Urine

Fluid intake and total daily excretion of an extensive list of urinary components following the first dive series (Dive I) are presented in Table I while Table II shows results for the same parameters following the second series (Dive II) performed 28 days later. In each table the test for significant difference from the average control values is based on a paired t-test analysis.

Although significant changes occurred in various parameters following both dives, there appeared to be no consistent pattern of response in any parameter, other than the ketosteroid excretion, that could be attributed to the pressure exposures. Similar ketosteroid responses were reported in an earlier study following exposures to increased air pressures of 2 and 7 ATA⁹. The reduced 17-ketosteroid excretion observed in both the previous and present studies is analogous to the decrease reported during periods of stress^{7,13} and would support the idea that a stress-like syndrome of several days duration follows exposure to increased air pressures. When the urinary data were calculated per gram of excreted creatinine, a method frequently employed to minimize the effect of volume fluctuation, there was again no consistent pattern of significant changes other than ketosteroid excretion. Moreover, Tappan et al.¹⁹ have shown that creatinine excretion is not stable under stress conditions, but can be correlated with ketosteroid alterations.

Table III compares the total excretion data obtained during the period of Dive I with those of Dive II for each of the parameters measured--i.e., control vs. control, 1 day post-dive vs. 1 day post-dive, etc. Again, significant changes are evaluated using the paired t-test. Since the means and standard errors of the mean for each of the parameters have been presented in Table I and II, they are not included in Table III and only those results which differ significantly at the 5% level or better for each parameter are indicated.

In predicting the post-dive effects of one exposure to 6.7 ATA on the responses to a similar dive after a 28 day interval, some residual effects on the excretory patterns might have been expected. However, the significant difference that occurs between the two control periods for sodium and urea nitrogen can unquestionably be related to their particular susceptibility to dietary intake. It may be noted that significant inter-dive differences for hydroxyproline, magnesium, potassium, and uric acid occur in the complete absence of any intra-dive changes. Phosphorus and Na/K ratio which exhibit inter-dive differences have noncorresponding within-dive alterations, and the changes observed in ketosteroid and solute excretion have coincidental intra-dive significant alterations.

Since inspection of Table III reveals a scattered and apparently unbiased pattern of inter-dive changes; we postulate that there is no real difference between the two dives and that any residual biochemical alterations follow-

ing Dive I have been attenuated and are not reflected in the responses to Dive II made 28 days later. Thus the two dives can be considered as separate unrelated exposures to 6.7 ATA and their values combined to yield a greater number of data points for each measurement period.

The results of this combination reveal significant post-dive changes in total 24-hour urinary excretion of calcium, potassium, keto- and hydroxysteroids, and total steroids. These data are presented in Figures 1 and 2. In comparing the present results with those from a previous study of exposure to 2 and 7 ATA in air⁹, it is evident that there is no pronounced decrease on the first post-dive day in urine volume with concomitant mineral and electrolyte decreases that characterized the earlier study. Decreases in urine volume following exposure to increased pressure have been reported after both short term and saturation dives in man^{1,9}. These post-dive decreases in urine volume have been interpreted as a rebound effect from the diuresis observed during periods of increased pressure and/or a compensatory correction of hypovolemia^{1,6,9,12,17}. The absence of a decrease in volume excreted on the first post-dive day in the present study seems to reflect the fact that the men were urged to make a concerted effort to increase their fluid intake on the day following the dive. The increase averaged 200 ml. Although potassium decreased significantly on the first post-dive day, the Na/K ratio was not altered inasmuch as sodium excretion also decreased albeit to a lesser extent.

TABLE I

Fluid Intake and Total 24 Hour Urinary Excretion Following
Exposure to Air at 6.7 ATA (Dive I). Mean \pm SEM.
Asterisk (*) Indicates $p \leq .05$ by Paired t-test. N=4

| PERIOD | # INT | EXC | HP | CA | PHOS | MG | NA | K |
|--------------------|----------------|---------------------|---------------|----------------|----------------|----------------|---------------|----------------|
| CONTROL | 1.651 0.239 | 1.273 0.055 | 37.83 3.40 | 0.145 0.015 | 1.065 0.164 | 0.075 0.004 | 159.1 21.0 | 67.55 10.22 |
| POST DV 1 DY | 2.418 0.459 | 1.573 0.262 | 41.67 7.70 | 0.133 0.039 | 0.887 0.237 | 0.072 0.014 | 139.1 54.2 | 52.53 8.01 |
| POST DV 2 DY | 1.818 0.239 | 1.092 0.275 | 23.67 5.43 | 0.111 0.019 | 0.705 0.129 | 0.069 0.014 | 154.3 27.9 | 64.14 14.53 |
| POST DV 3 DY | 2.538 0.506 | 1.610 0.142 | 41.37 3.96 | 0.155 0.041 | 0.902 0.196 | 0.088 0.009 | 231.8 54.2 | 82.93 12.43 |
| POST DV 4 DY | 1.555 0.258 | 1.593 0.106 * | 41.62 0.93 | 0.152 0.022 | 0.942 0.097 | 0.086 0.011 | 241.5 22.4 | 80.43 10.07 |
| POST DV 5-6 DY | 1.721 0.157 | 1.339 0.059 | 41.39 5.64 | 0.185 0.034 | 0.990 0.271 | 0.086 0.011 | 190.3 24.6 | 70.66 7.41 |
| POST DV 7-8 DY | 1.724 0.319 | 1.144 0.278 | 29.01 6.63 | 0.108 0.018 | 0.695 0.087 | 0.058 0.013 | 137.5 21.4 | 57.05 11.77 |
| POST DV 9-10 DY | 1.564 0.225 | 1.294 0.162 | 39.34 3.13 | 0.166 0.034 | 1.099 0.147 | 0.088 0.009 | 218.8 43.7 | 72.20 9.96 |

#INT = fluid intake, l; EXC = urinary excretion, l; HP = hydroxy-
proline, mg; CA = calcium, gm; PHOS = inorganic phosphorus, gm;
MG = magnesium, gm; NA = sodium, mEq; K = potassium, mEq.

TABLE I

Fluid Intake and Total 24 Hour Urinary Excretion Following
Exposure to Air at 6.7 ATA (Dive I). Mean \pm SEM.
Asterisk (*) Indicates $p \leq .05$ by Paired t-test. N=4
(continued)

| PERIOD | # UN | UA | CRT | KS | OHS | OSM | NA/K | TSTR |
|--------------------|---------------------|----------------|---------------------|--------------------|--------------------|---------------------|---------------------|--------------------|
| CONTROL | 8.793 0.268 | 0.963 0.054 | 1.793 0.135 | 19.28 0.61 | 17.05 1.15 | 0.830 0.075 | 3.094 0.318 | 36.34 1.49 |
| POST DV 1 DY | 5.313 0.571 * | 0.925 0.127 | 1.258 0.163 * | 18.96 2.33 | 19.39 3.41 | 0.734 0.195 | 4.222 1.445 | 38.35 4.57 |
| POST DV 2 DY | 4.738 1.114 * | 0.728 0.189 | 1.060 0.187 | 15.34 3.59 | 16.80 3.57 | 0.795 0.151 | 2.678 0.697 | 32.15 6.90 |
| POST DV 3 DY | 7.680 1.242 | 0.980 0.121 | 1.512 0.162 * | 16.81 1.46 | 20.73 2.03 | 1.102 0.171 | 4.501 0.722 | 37.54 3.37 |
| POST DV 4 DY | 7.946 0.518 | 1.145 0.073 | 1.645 0.032 | 18.14 2.22 | 22.85 2.55 | 1.042 0.044 | 4.822 0.387 * | 40.99 4.65 |
| POST DV 5-6 DY | 8.510 1.122 | 0.984 0.180 | 1.695 0.284 | 17.91 0.46 | 22.94 1.00 * | 1.018 0.119 | 3.757 0.255 | 40.85 1.15 * |
| POST DV 7-8 DY | 5.826 0.840 * | 0.926 0.152 | 1.313 0.214 | 13.83 2.95 | 18.09 4.28 | 0.755 0.112 | 2.831 0.527 | 31.92 7.14 |
| POST DV 9-10 DY | 7.653 0.301 * | 1.200 0.207 | 1.616 0.115 | 10.69 0.80 * | 15.75 1.70 | 1.067 0.123 * | 3.967 0.774 | 26.43 2.39 |

#UN = urea nitrogen, gm; UA = uric acid, g; CRT = creatinine, gm;
KS = ketosteroids, mg; OHS = hydroxysteroids, mg; OSM = osmoles;
NA/K = sodium/potassium ratio; TSTR - total steroids, mg.

TABLE II

Fluid Intake and Total 24 Hour Urinary Excretion Following
Second Exposure to Air at 6.7 ATA (Dive II) Performed 28
Days after Dive I. Mean \pm SEM
Asterisk (*) Indicates $p \leq .05$ by Paired t-test. N=4

| PERIOD | # INT | EXC | HP | CA | PHOS | MG | NA | K |
|--------------------|----------------|----------------|---------------|----------------|----------------|----------------|---------------|----------------|
| CONTROL | 2.833 0.451 | 1.354 0.171 | 29.96 4.60 | 0.201 0.041 | 1.300 0.219 | 0.111 0.018 | 200.7 31.6 | 74.42 9.81 |
| POST DV 1 DY | 1.735 0.296 | 1.415 0.200 | 30.82 4.51 | 0.205 0.076 | 1.351 0.232 | 0.111 0.022 | 171.7 40.9 | 67.73 7.93 |
| POST DV 2 DY | 1.885 0.157 | 1.385 0.089 | 27.45 3.19 | 0.163 0.037 | 1.017 0.190 | 0.102 0.014 | 177.1 18.1 | 62.34 10.01 |
| | | | | * | | | | |
| POST DV 3 DY | 1.715 0.108 | 1.225 0.173 | 25.48 4.48 | 0.182 0.038 | 1.356 0.229 | 0.102 0.008 | 182.9 43.3 | 70.21 12.71 |
| POST DV 4 DY | 1.880 0.223 | 1.108 0.137 | 30.36 3.85 | 0.162 0.050 | 1.661 0.243 | 0.093 0.019 | 159.6 27.1 | 68.73 11.22 |
| | | | | * | | | | |
| POST DV 5-6 DY | 1.678 0.054 | 1.391 0.133 | 27.52 1.89 | 0.203 0.047 | 1.462 0.164 | 0.110 0.016 | 163.4 10.5 | 66.08 8.66 |
| POST DV 7-8 DY | 1.829 0.214 | 1.428 0.224 | 27.03 1.98 | 0.155 0.027 | 1.183 0.024 | 0.098 0.017 | 185.7 20.4 | 70.28 8.33 |
| POST DV 9-10 DY | 1.901 0.256 | 1.296 0.201 | 25.57 3.29 | 0.176 0.040 | 1.211 0.162 | 0.098 0.018 | 146.5 35.0 | 62.57 15.28 |
| | | | | * | | | * | |

#INT = fluid intake, l; EXC = urinary excretion, l; HP = hydroxyproline, mg;
CA = calcium, gm; PHOS = inorganic phosphorus, gm; MG = magnesium, gm;
NA = sodium, mEq; K = potassium, mEq.

(continued on facing page)

TABLE II

Fluid Intake and Total 24 Hour Urinary Excretion Following
Second Exposure to Air at 6.7 ATA (Dive II) Performed 28
Days after Dive I. Mean \pm SEM.

Asterisk (*) Indicates $p \leq .05$ by Paired t-test. N=4
(continued)

| PERIOD | # UN | UA | CRT | KS | OHS | OSM | NA/K | TSTR |
|--------------------|---------------------|----------------|----------------|--------------------|--------------------|----------------|---------------------|--------------------|
| CONTROL | 4.706 0.715 | 0.911 0.179 | 1.699 0.254 | 18.43 2.32 | 18.98 1.12 | 0.931 0.140 | 3.697 0.415 | 37.41 3.44 |
| POST DV 1 DY | 5.545 0.793 * | 0.920 0.223 | 1.737 0.231 | 10.10 1.67 * | 18.60 2.20 | 0.922 0.127 | 3.875 1.153 | 28.70 3.75 * |
| POST DV 2 DY | 5.273 0.736 | 0.996 0.251 | 1.600 0.217 | 8.99 1.27 * | 19.38 3.33 | 0.874 0.082 | 4.138 0.524 | 28.37 4.44 |
| POST DV 3 DY | 5.633 0.921 | 0.804 0.245 | 1.737 0.254 | 10.11 1.38 | 20.02 3.29 | 0.934 0.144 | 3.199 0.722 | 30.13 4.67 |
| POST DV 4 DY | 7.255 1.610 | 0.915 0.183 | 2.159 0.346 | 13.00 1.62 * | 23.96 3.21 | 0.953 0.173 | 2.565 0.313 * | 36.96 4.32 |
| POST DV 5-6 DY | 7.512 1.609 | 0.623 0.166 | 2.045 0.252 | 11.27 0.47 * | 24.65 1.46 * | 0.993 0.104 | 3.604 0.575 | 35.92 1.27 |
| POST DV 7-8 DY | 5.590 0.375 | 0.855 0.062 | 1.726 0.094 | 11.90 0.32 | 24.02 3.91 | 0.948 0.072 | 3.772 0.484 | 35.92 4.67 |
| POST DV 9-10 DY | 5.007 0.537 | 0.676 0.161 | 1.661 0.111 | 11.14 0.73 * | 18.56 1.05 | 0.852 0.135 | 3.082 0.502 | 30.00 1.69 * |

#UN = urea nitrogen, gm; UA = uric acid, g; CRT = creatinine, gm;
KS = ketosteroids, mg; OHS = hydroxysteroids, mg; OSM = osmoles;
NA/K = sodium/potassium ratio; TSTR = total steroids, mg.

TABLE III

Significant Differences Observed in Total Urinary Excretion Between Two Dives to 6.7 ATA
28 Days Apart. Paired t-tests compare each time period; $p \leq .05$ is indicated by an
Asterisk (*)

| PERIOD # | INT | EXC | OSM | HP | CA | PHOS | MG | Na | K | Na/K | UN | UA | CRT | KS | OHS | TSTR |
|---------------|-----|-----|-----|----|----|------|----|----|---|------|----|----|-----|----|-----|------|
| Control | | | | | | | | * | | | * | | | | | |
| POST DIVE DAY | | | | | | | | | | | | | | | | |
| 1 | | | | | | * | | | | | | | | * | | |
| 2 | | | | | | | | | | | | | | | | |
| 3 | | | | * | | | | | | | | | | | | |
| 4 | * | | | * | | | | | | * | | | | | | |
| 5 - 6 | | | | | | * | | * | * | * | | * | * | * | | * |
| 7 - 8 | | | | | | * | | | | | | | | | | |
| 9 - 10 | * | | * | * | | | | | | | | * | | | * | |

INT = Fluid intake
OSM = Osmoles
CA = Calcium
MG = Magnesium
K = Potassium
UN = Urea nitrogen
CRT = Creatinine
OHS = Hydroxysteroids

EXC = Urinary excretion
HP = Hydroxyproline
PHOS = Inorganic phosphorus
Na = Sodium
Na/K = Sodium/potassium ratio
UA = Uric acid
KS = Ketosteroids
TSTR = Total steroids

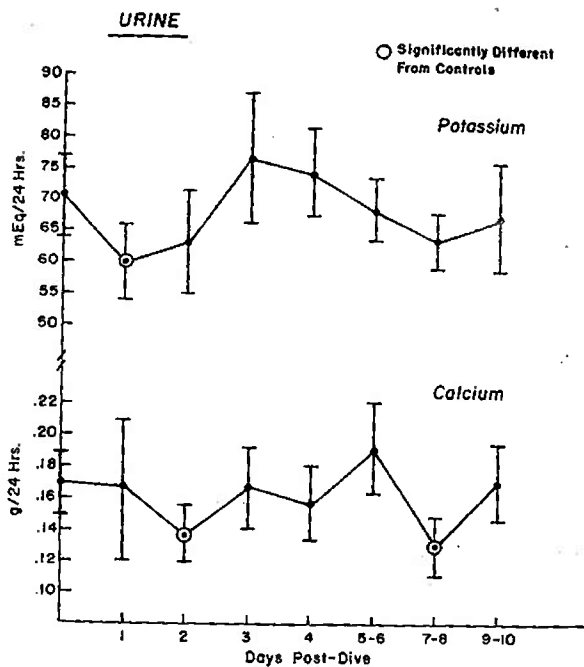


Fig. 1. Total urinary excretion/24 hours. Combined data of Dives I and II for potassium and calcium. Mean \pm SEM. N = 8.

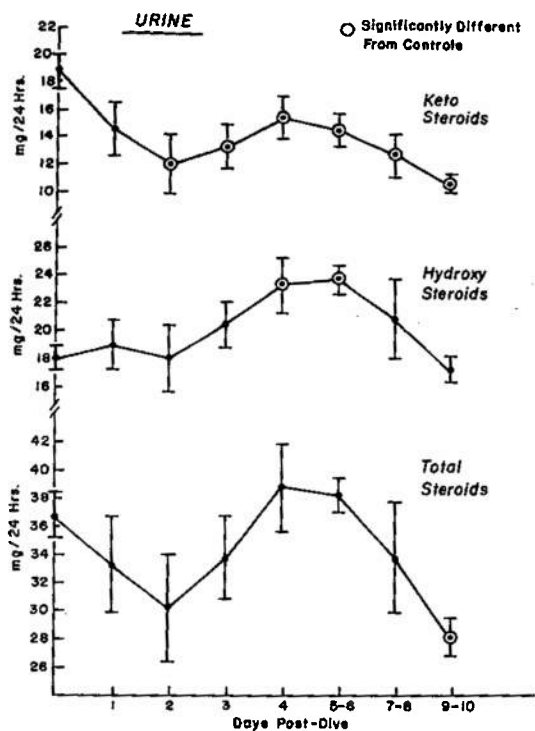


Fig. 2. Total urinary excretion/24 hours. Combined data of Dives I and II for ketosteroids, hydroxysteroids, and total steroids. Mean \pm SEM. N = 8.

The decrease in ketosteroid excretion, nonsignificant in Dive I and significant in Dive II, when combined into a single study demonstrates a significant change (Figure 2). This further supports earlier observations that an extended period of time is required for biochemical equilibria to become re-established following exposure to compression/decompression, and emphasizes that recovery from multifactor stress requires more time than is needed to induce the stress.

Serum

The data recorded from the sera obtained following Dives I and II are presented in Tables IV and V, respectively. Of the parameters measured, serum ionized calcium levels are significantly lowered following the initial exposure to 6.7 ATA (Table IV). This decrease occurred by one hour and lasted through one day post-dive. This phenomenon was not observed following Dive II performed 28 days later. Changes in total calcium were observed after both dives on the 3rd post-dive day; a significant decrease following Dive I and a significant increase after Dive II. Elevations in serum magnesium occurred on the 7th post-dive day following both exposures. An increase in magnesium was also observed on the 3rd post-dive day of Dive II as well as an increase in potassium on the 5th day. Again intradive alterations appear to be unrelated to inter-dive changes and indicates that no real difference exists between the two dives that could be attributed to residual effects from Dive I. Thus these data from Dive I and Dive II have also been combined. The combined data reveal significant post-dive changes

TABLE IV
 Effect of Initial Exposure to Air at 6.7 ATA (Dive I) on
 Serum Minerals and Electrolytes. Mean \pm SEM.
 Asterisk (*) Indicates $p \leq .05$ by Paired t-test. N=4

| PERIOD | #CA++ | CA | PHOS | MG | NA | K | MOSM | UN |
|-----------------|---------------------|----------------|----------------|----------------|--------------|---------------------|--------------|---------------|
| CONTROL | 5.419 0.053 | 9.167 0.159 | 3.658 0.539 | 1.908 0.059 | 134.2 0.3 | 3.933 0.126 | 295.9 3.7 | 16.77 1.75 |
| POST DV 1 HR | 5.143 0.091 * | 9.375 0.342 | 3.650 0.433 | 2.005 0.040 | 133.9 0.7 | 3.975 0.118 | 294.5 3.6 | 18.00 0.00 |
| POST DV 1 DY | 5.230 0.087 * | 9.325 0.175 | 3.725 0.477 | 1.977 0.068 | 135.0 1.1 | 4.350 0.206 * | 295.5 2.1 | 13.37 1.89 |
| POST DV 3 DY | 5.292 0.122 * | 9.025 0.180 | 3.225 0.437 | 1.868 0.053 | 133.6 0.5 | 3.875 0.085 | 296.5 5.6 | 15.12 1.69 |
| POST DV 5 DY | 5.410 0.033 | 9.275 0.193 | 3.925 0.390 | 1.870 0.046 | 134.7 0.6 | 4.125 0.193 | 301.0 3.8 | 19.75 0.75 |
| POST DV 7 DY | 5.298 0.150 * | 9.025 0.246 | 3.625 0.453 | 1.953 0.065 | 134.3 0.3 | 3.925 0.272 | 298.2 3.4 | 13.75 2.13 |

CA++ = ionized calcium, mg%
 CA = total calcium, mg%
 PHOS = inorganic phosphorus, mg%
 MG = magnesium, mg%
 NA = sodium, mEq/l
 K = potassium, mEq/l
 MOSM = milliosmoles/l
 UN = urea nitrogen

TABLE V
 Effect of a Second Exposure to Air at 6.7 ATA (Dive II)
 Performed 28 Days after Dive I on Serum Minerals and
 Electrolytes. Mean \pm SEM.
 Asterisk (*) Indicates $p \leq .05$ by Paired t-test. N=4

| PERIOD | #CA++ | CA | PHOS | MG | NA | K | MOSM | UN |
|-----------------|----------------|---------------------|----------------|---------------------|--------------|---------------------|--------------|---------------|
| CONTROL | 5.028 0.055 | 9.092 0.104 | 3.167 0.202 | 1.733 0.028 | 135.1 0.9 | 3.833 0.190 | 257.8 3.3 | 19.42 0.81 |
| POST DV 1 HR | 4.760 0.179 | 9.125 0.132 | 3.425 0.197 | 1.843 0.077 | 133.2 0.4 | 3.775 0.125 | 286.7 4.2 | 19.37 0.85 |
| POST DV 1 DY | 4.973 0.127 | 8.733 0.406 | 3.300 0.404 | 1.733 0.072 | 134.2 0.2 | 3.933 0.145 | 281.3 4.9 | 19.33 1.09 |
| POST DV 3 DY | 4.860 0.116 | 9.275 0.111 * | 3.225 0.253 | 1.795 0.037 * | 134.7 0.4 | 4.125 0.197 | 291.0 3.8 | 17.75 1.93 |
| POST DV 5 DY | 5.050 0.144 | 9.150 0.499 | 2.650 0.144 | 1.807 0.096 | 134.3 0.9 | 4.225 0.125 * | 233.8 6.7 | 16.62 1.57 |
| POST DV 7 DY | 4.850 0.132 | 9.475 0.366 | 3.225 0.259 | 1.820 0.050 * | 133.7 0.3 | 4.525 0.232 | 290.5 4.0 | 19.25 0.72 |

CA++ = ionized calcium, mg%
 CA = total calcium, mg%
 PHOS = inorganic phosphorus, mg%
 MG = magnesium, mg%
 NA = sodium, mEq/l
 K = potassium, mEq/l
 MOSM = milliosmoles/l
 UN = urea nitrogen, mg%

in serum ionized calcium, magnesium, and potassium, and these results are presented in Figure 3. Although post-dive blood data were not available from previous dives to 2 and 7 ATA using air⁹, other studies of biochemical responses of men to simulated air dives to 100 feet¹⁸ have shown oscillations in several serum constituents. In the latter studies, serum potassium decreased significantly immediately post-dive followed by a gradual return to control values by the fifth post-dive day. On the other hand, serum potassium levels rose significantly on the third and fifth days in the present study while serum ionized calcium decreased significantly one hour and one day after exposure.

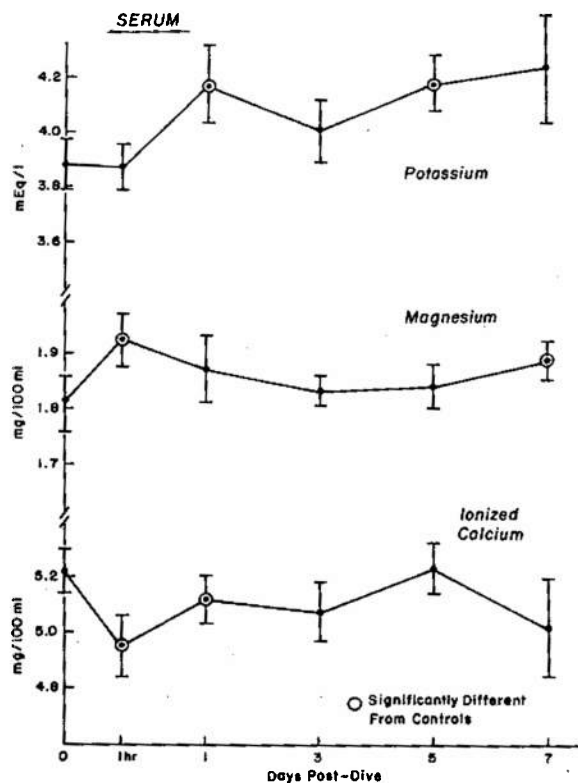


Fig. 3. Combined data of Dives I and II for serum concentrations of potassium, magnesium, and ionized calcium. Mean \pm SEM. N = 8.

The comparison of results among several different types of single dives using air, or between two essentially identical dives with at least 28 day inter-dive intervals, leads one to conclude that within the protocol of "safe" dives, individual variability of the divers, or subtle differences in diving procedures such as compression rate or work performed, profoundly alters the kind and/or direction of the biochemical changes observed. This is not to say, however, that minimal or sub-threshold alterations in biochemical parameters are not cumulative and may be detrimental to the health of the divers. The development of aseptic osteonecrosis occasionally observed in divers following multiple pressure exposures^{4,11} may well reflect such phenomena. Repetitive dives with short inter-dive intervals may shed some light on possible cumulative biochemical effects of hyperbaric exposure.

REFERENCES

- Alexander, W.C., Leach, C.S., Fischer, C.L., Lambertsen, C.J. and Johnson, P.C. Hematological, biochemical and immunological studies during a 14-day continuous exposure to 5.2% O₂ in N₂ at pressure equivalent to 100 FSW (4 ata). *Aerosp Med* 44: 850-854, 1973.
- Bennett, P. B. and Hayward, A. J. Electrolyte imbalance as the mechanism for inert gas narcosis and anesthesia. *Nature* 213: 938-939, 1967.

3. Bennett, P.B. and Gray, S.P. Changes in human urine and blood chemistry during a simulated oxygen-helium dive to 1500 feet. Aerosp Med 41: 868-874, 1971.
4. Elliott, D.H. Decompression inadequacy in relation to aseptic bone necrosis in divers. Presented Royal Soc of Med, London, March 4, 1971.
5. Few, J.D. A method for the analysis of urinary 17-hydroxycorticosteroids. J Endocrinol 22: 31-46, 1961.
6. Frattali, V., Raymond, L., Robertson, R., and Quesada, M. Nutritional aspects among Naval personnel chronically exposed to wet/dry simulated dives at pressures attaining the equivalent to 1600 feet below sea level. Fed Proc 33: 698, 1974.
7. Hale, H.B., Storm, W.F., Goldzieher, J.W., Hartman, B.O., Miranda, R.E., and Hosenfield, J.M. Physiological cost in 36- and 48-hour simulated flights. Aerosp Med 44: 871-881, 1973.
8. Heyder, E. and Tappan, D.V. Mineral and electrolyte responses following severe decompression stress. NAVSUBMEDRSCHLAB Report #743, 1973.
9. Heyder, E. and Tappan, D.V. Excretion of minerals and nitrogen metabolites following exposure to increased air pressures (2 or 7 ATA). NAVSUBMEDRSCHLAB Report #765, 1973.
10. Hosley, H.F., Olson, K.B., Horton, J., Michelsen, P. and Atkins, R. Automated analysis of urinary hydroxyproline for cancer research. Technicon International Congress: Advances in Automated Anal. 1: 104-110, 1969.
11. Kawashima, M., Torisu, T., Hayashi, K., and Kamo, Y. Avascular bone necrosis in Japanese diving fishermen. 5th Int Hyperbaric Congress Proc 855-862, 1973.
12. MacInnis, J.B. and Bond, G.F. Saturation diving: Man-in-Sea and SeaLab. In: The Physiology and Medicine of Diving and Compressed Air Work. P.B. Bennett and D.H. Elliott, eds. London. Bailliere, Tindall and Cassell, 1969, pp. 505-523.
13. Mason, J.W. Tolson, W.W. Robinson, J.A. Brady, J.V. Tolliver, G.A. and Johnson, T.A. Urinary androsterone, etiocholanolone, and dehydroepiandrosterone responses to 72-hour avoidance sessions in the monkey. Psychosom Med 30: 710, 1968.
14. Metcalf, M.G. A rapid method for measuring 17-hydroxycorticosteroids in urine. J. Endocrinol 26: 415-423, 1963.
15. Philp, R.B., Ackeles, K.N., Inwood, M.J., Livingston, S.D.,

- Achimastos, A., Binns-Smith, M. and Radomski, M. W. Changes in the hemostatic system and blood and urine chemistry of human subjects following decompression from a hyperbaric environment. Aerosp Med 43: 498-505, 1972.
16. Radomski, M.W. and Bennett, P.B. Metabolic changes in man during short exposure to high pressure. Aerosp Med 41: 309-313, 1970.
17. Schaefer, K.E., Carey, C.R. and Dougherty, J.H. Jr. Pulmonary gas exchange and urinary electrolyte excretion during saturation-excursion diving to pressures equivalent to 800 and 1000 feet of seawater. Aerosp Med 41: 856-864, 1970.
18. Tappan, D.V. and Heyder, E. Biochemical responses of men to simulated air dives of 100 feet. NAVSUBMEDRSCHLAB Report #774, 1974.
19. Tappan, D.V., Madden, R.O. and Jacey, M.J. Urinary indicators of stress: Effects of exposure to simulated sonar noise for 8-23 days. NAVSUBMEDRSCHLAB Report #766, 1973.
20. U.S. Navy Diving Manual, NAVSHIPS 0994-001-9010, Navy Department, Washington, D.C., March 1970.
21. Zak, B., Epstein, E. and Kraushaar, L.A. Semi-automated determination of 17-ketosteroids and 17-ketogenic steroids. In: Automation in Analytical Chemistry. L.T. Skeggs, ed., New York: Mediad, 1966, pp. 336-340.

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| 13. ABSTRACT <p>This study concerns an evaluation of the possible effects of repetitive diving and long-term effects of compression/decompression on distribution of, or loss of minerals, electrolytes, and protein metabolites in men subjected to standard Navy diving procedures.</p> <p>Urinary minerals, electrolytes, nitrogen metabolites and steroids, as well as serum minerals and electrolytes, were measured in Navy divers following short exposures to air at 6.7 ATA. A comparison was made between two similar dives performed 28 days apart.</p> <p>Increased fluid intake apparently prevented the reduction in urinary output, with its concomitant decreases in mineral and nitrogen metabolite excretion, that had been previously observed during the first post-dive day. Urinary ketosteroid excretion was slightly reduced after the first dive and decreased significantly for up to 10 days following the second hyperbaric exposure. This observation suggests prolonged adrenal responses to the stresses of compression/decompression.</p> <p>Although serum ionized and total calcium, and magnesium levels showed considerable variability in this diving protocol, no consistent pattern of intra- or inter-dive variability was observed. These and the other serum parameters measured indicate little or no carryover from the first to the second dive series performed 28 days later.</p> | | | |

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| Dysbaric stress | | | | | | |
| Mineral metabolism | | | | | | |
| Urinary metabolites | | | | | | |

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