

**NAVAL SUBMARINE MEDICAL  
RESEARCH LABORATORY  
SUBMARINE BASE, GROTON, CONN.**



REPORT NUMBER 877

**EFFECTS ON SERUM CONSTITUENTS  
AND URINARY METABOLITE EXCRETION  
OF REPEATED COMPRESSED AIR DIVES**

by

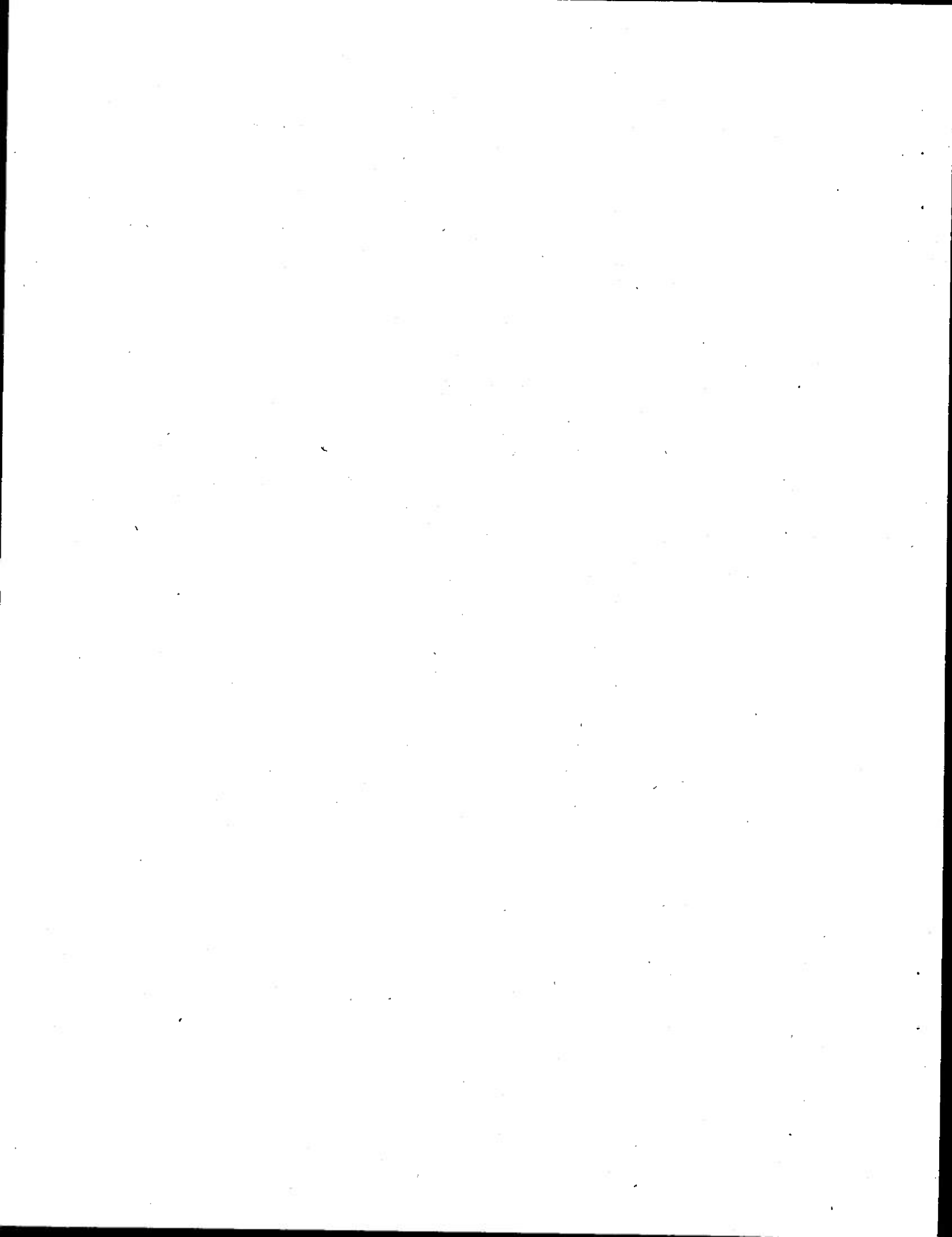
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**Naval Medical Research and Development Command  
Research Work Unit MF51.524.014-9016**

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EFFECTS ON SERUM CONSTITUENTS AND URINARY METABOLITE EXCRETION  
OF REPEATED COMPRESSED AIR DIVES

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## SUMMARY PAGE

### THE PROBLEM

To investigate the effects of short hyperbaric exposures repeated at frequent intervals for evaluation of cumulative effects on metabolic processes.

### FINDINGS

The responses of serum components indicate that mild stresses result from brief (45-50 min) repeated exposures to air at 6.7 ATA. In most cases, when 72 hours of recovery intervened between dives, temporarily disrupted serum metabolite levels returned to control values before the next dive. Five daily dives, on the other hand, produced cumulative residual serum changes that were corrected or perhaps overcorrected about 7 days following the last dive.

No diuresis or changes in glomerular filtration rate were demonstrated to result from the repeated sequences of compression and decompression.

### APPLICATION

No strong evidence was obtained during these experiments to contradict the working assumptions that brief dives to at least 6.7 ATA in air may be made on a daily basis. However, since biochemical responses do occur and continue for several days following such hyperbaric exposure, careful attention to details of allowable exposure times and proper decompression routines must be paid in order to perform repeated short-term dives safely.

### ADMINISTRATIVE INFORMATION

This investigation was conducted as part of Naval Medical Research and Development Command Research Work Unit MF51.524.014-9016 - "Effects on serum constituents and urinary metabolite excretion of repeated compressed air dives". The present report is Number 5 on this work unit. It was submitted for review on 10 Aug 1978, approved for publication on 29 Aug 1978, and designated as NSMRL Report No. 877.

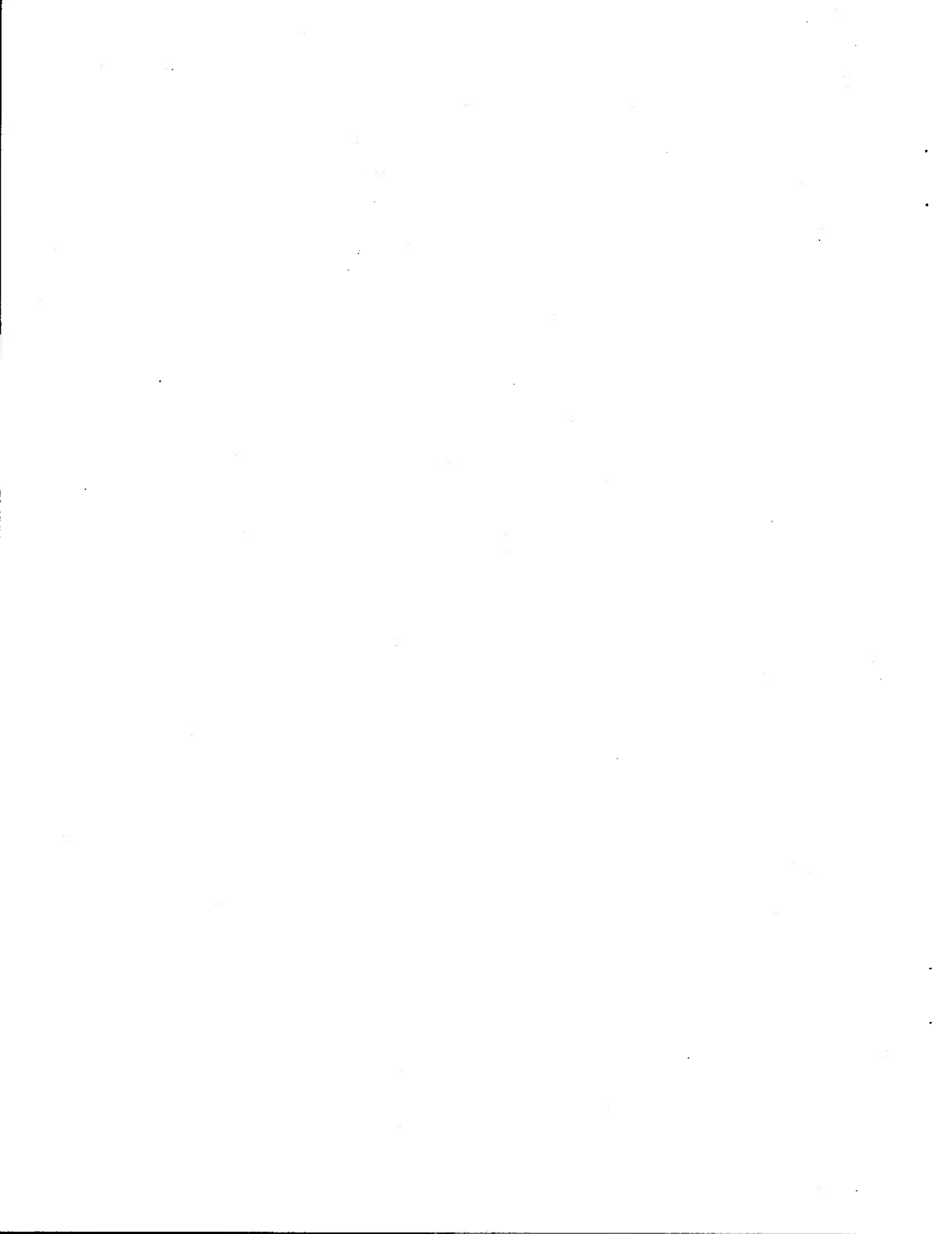
## ABSTRACT

Eighteen qualified U. S. Navy divers underwent either of two series of dives to 6.7 ATA for 45-50 minutes in compressed air in a dry chamber: 4 dives at 3-day intervals or 5 dives performed daily. Serum and urinary components were monitored before and after the dives to compare the biologic influence of repeated diving with the known effects of hyperbaric saturation exposures.

No diuresis or changes in glomerular filtration rate were demonstrated to result from the repeated sequences of pressurization and decompression. Biochemical evidence supports the previous finding of a hemodilution resulting from repeated short-term pressure exposures. The increasing extent of metabolic changes during the first few dives leads to the conclusion that adaptation to repeated dives may begin to play a role after two or three dives particularly when performed at three-day intervals. While no cumulative effects beyond those associated with 2-3 dives are apparent when dives are performed with a few days intervening, limited biologic changes occur when dives are performed daily and seem to require an extended recovery period to allow metabolic processes to return to a state of predive equilibrium.

Repeated diving  
Compressed air diving

Serum constituents (changes)  
Urinary metabolites



metabolic processes.

## MATERIALS AND METHODS

The studies described were performed in the Naval Submarine Medical Research Laboratory's dry, man-rated 473 M3 hyperbaric chamber maintained at 25.5°C. The divers' body temperature, however, were not monitored. Each simulated dive commenced at 0830 exposing the divers to air under pressure equivalent to 188 fsw (6.7 ATA). The men, all qualified navy divers, were compressed at a rate of 75 ft/min. Bottom time was 45-50 minutes and decompression was performed according to standard U.S. Navy diving procedures using the table for a 50-minute exposure to air at 190 feet (U.S. Navy Diving Manual, 1970).

The study was divided into two experiments: (a) a series of four dives at 3-day intervals and (b) a series of five daily dives. The dives were performed using 7 and 11 different subjects, respectively. However, since three of the divers in series B required recompression for symptoms of dysbarism after their first or second dives, they were omitted from the remainder of the study. Prior to his first dive, each man collected five consecutive 24-hour, acidified urine samples to serve as controls. Similar urine collections were also made during the experimental periods and for up to 10 days after completion of the final dive in each series. Since no individual voidings were collected during the dives, the urine samples for the first post-dive days contained the urine produced during the dive periods. Individual fluid intake logs were maintained by the subjects during the studies.

To provide serum control data, three blood samples were drawn from

## INTRODUCTION

Exposure to increased pressure and decompression from elevated pressures result in alteration in blood and urinary minerals, electrolytes, protein metabolites, and hormones (Alexander, et al, 1973; Radomski and Bennett, 1970).

Severely decompressed rats have been shown to exhibit mineral and electrolyte alterations for up to 5 days post-decompression (Heyder and Tappan, 1973A). In men exposed to air at 2 or 7 atmospheres pressure for 45 minutes, changes were evident in urinary minerals, electrolytes, and protein metabolites for at least 4 days (Heyder and Tappan, 1973B). Similar results have been observed for up to 7 days following simulated dives in air to 100 feet of sea water (fsw) for 60 minutes (Tappan and Heyder, 1974).

Although exposures to hyperbaric environments result in biochemical alterations of several days' duration, when the relationship between interdive interval and the cumulative effect of repeated pressurizations was studied by Heyder and Tappan (1974) using a 28-day interval between exposure to air at 6.7 atmospheres, no apparent biochemical carry-over for this extended period was observed. On the other hand, certain potential effects of diving such as aseptic bone necrosis appear to accumulate and may be related to the frequency and type of pressure experience (Elliott, 1971; Kawashima, et al, 1973). The present study was performed to investigate the effects of short exposure at frequent intervals on metabolites, electrolytes, and fluid shifts for evaluation of cumulative effects on

the subjects prior to their initial dives, after overnight fasts. In series A, blood was withdrawn one hour post-surfacing, and on the first and third days following each dive with the samples for the third post-dive taken just prior to the next dive in the series. In series B, blood was taken daily one hour after completion of each dive. Blood specimens were also obtained one hour and 1, 3, 5, and 7 days following the final dive in both series. With the exception of the one-hour post-dive samples, blood specimens were taken at 0730.

Calcium was measured by atomic absorption spectrometry while sodium and potassium levels were determined by flame photometry. Phosphorus, urea nitrogen, uric acid, proteins, and creatinine measurements were made using technicon auto-analyzer techniques. Serum cortisol was measured by competitive protein binding using schwarz/mann 3H cortisol kits. Freezing point depression was used to determine osmolality.

The significance of changes occurring during the experimental periods were determined by paired-T analysis in which the data for each subject was compared against mean values for that man obtained during the control period.

## RESULTS

In order to concisely present the voluminous data collected, figures 1 and 2 show the control values for each parameter with any statistically significant changes from those control values indicated by arrows. The changes depicted are at the 5% level or better with mean control values generally falling within the accepted ranges of normality.

In figure 1, it may be seen that the most significant disruptions in serum metabolite levels tended to occur soon after return of the subjects to one atmosphere pressure following pressuring action. The responses of the individual serum components indicate mild stresses occur rather than consistent major biochemical changes resulting from the brief dives. In most cases when 72 hours of recovery intervened between the dives (Series A), the temporarily disrupted serum metabolites' levels returned to control values before the next dive.

Figure 2 presents data on changes from control values for daily excretion of metabolic constituents into urine throughout the experimental periods. The trend most apparent from these data is the reduced excretion of several components beginning shortly after the first dives of each series and which extended through the dive periods and continued for several days following the dives. The most consistent changes seem to occur among the nitrogenous metabolites, urea nitrogen and uric acid, with similar alterations evident for osmolality.

The excretory volumes (Table 1) reveal a remarkable correspondence between the incidence of metabolic shifts evident in the serum and urine chemistry data (figures 1 and 2) and the total quantities of urine produced. Note, for example, the data for the second days after dives 1 and 3, (experiment A) when both solute excretion and urinary volume decreased markedly. In the first three of the four dives of series A, volumes were higher on the first day following each of the dives than on the next two days. When dives were repeated daily in series B no similar phenomenon occurred. No consistent changes in creatinine or osmolal



FIGURE 1  
SIGNIFICANT DIFFERENCES OBSERVED IN SERUM CONSTITUENTS OF REPEATED DIVES TO 6.7 ATA  
PAIRED T-TESTS DETERMINES DIFFERENCE FROM CONTROL VALUES

PERIOD	#CA	PHOS	NA	K	MG	CRT	UN	UA	OSM	TP	ALB	GLOB	A/G	BILI
CONTROL	10.01	3.41	139.3	4.28	1.98	.988	16.1	5.36	.300	7.36	4.45	2.87	1.53	.58
A	9.68	3.47	141.3	4.07	-	1.090	13.0	6.12	.279	7.32	4.77	2.57	1.87	.43
1st DIVE POST-DIVE														
1 HOUR	↑						↓	↓				↓	↑	↑
1 DAY			↓											
3 DAYS			↓											
2nd DIVE POST-DIVE														
1 HOUR	↑						↓	↑	↓		↑	↓		
1 DAY							↓							
3 DAYS	↑													
3rd DIVE POST-DIVE														
1 HOUR	↑	↓					↓			↑	↑	↑	↓	↑
1 DAY	↑				↑									
3 DAYS											↓			
4th DIVE POST-DIVE														
1 HOUR	↑								↓	↑	↑			
1 DAY														
3 DAYS	↓										↓			
5th DIVE POST-DIVE														
1 HOUR									↓				↑	↑
1 DAY														
3 DAYS		↓				↑			↓				↑	
POST-DIVE														
5 DAYS			↓			↑								
7 DAYS	↑			↑		↑	↑			↑	↑		↑	↑

# CA = CALCIUM, MG/DL  
PHOS = INORGANIC PHOSPHORUS, MG/DL  
NA = SODIUM, mEq/L  
K = POTASSIUM, mEq/L  
MG = MAGNESIUM, MG/DL  
CRT = CREATININE, MG/DL  
UN = UREA NITROGEN, MG/DL

UA = URIC ACID, MG/DL  
OSM = OSMOLALITY, OSM/KG H<sub>2</sub>O  
TP = TOTAL PROTEIN, G/DL  
ALB = ALBUMIN, G/DL  
GLOB = GLOBULIN, G/DL  
A/G = ALBUMIN/GLOBULIN RATIO  
BILI = BILIRUBIN, MG/DL

\* A: 4 DIVES - 3 DAYS APART  
B: 5 DIVES - DAILY

↑ ↓ } P < .05  
↑ ↓ }

**FIGURE 2**  
**SIGNIFICANT DIFFERENCES OBSERVED IN TOTAL URINARY CONSTITUENTS OF REPEATED DIVES TO 6.7 ATA**  
**PAIRED T-TESTS DETERMINE DIFFERENCE FROM CONTROL VALUES**

PERIOD	#A	#B	#INT	EXC	HP	CA	P	MG	NA	K	NA/K	UN	UA	CRT	OSM	KS											
CONTROL	2.140	2.203	1.525	1.412	35.9	24.2	.171	.178	1.088	1.255	.145	205	188	83.0	64.4	2.63	3.14	15.5	20.8	.787	1.111	1.936	1.148	.982	18.81	20.44	
1st DIVE POST-DIVE																											
1 DAY						↓	↓	↓	↓	↓				↓							↓			↓			
2 DAYS				↓																			↓				
3 DAYS																											
2nd DIVE POST-DIVE																											
1 DAY						↓																	↓		↓		
2 DAYS						↓																	↓		↓		↓
3 DAYS																											
3rd DIVE POST-DIVE																											
1 DAY																											
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5th DIVE POST-DIVE																											
1 DAY																											
2 DAYS																											
3 DAYS																											
POST DIVE																											
4 DAYS																											
5-6 DAYS																											
7-8 DAYS																											
9-10 DAYS																											

# INT = INTAKE, L

EXC = EXCRETION, L

HP = HYDROXYPROLINE, MG

CA = CALCIUM, G

P = INORGANIC PHOSPHORUS, G

MG = MAGNESIUM, G

NA = SODIUM, mEq

K = POTASSIUM, mEq

NA/K = SODIUM POTASSIUM RATIO

UN = UREA NITROGEN, G

UA = URIC ACID, G

CRT = CREATININE, G

OSM = OSMOLALITY, OSMOLES

KS = KETOSTEROIDS, MG

ALL VALUES PER 24 HOURS

\* A: 4 DIVES - 3 DAYS APART

B: 5 DIVES - DAILY

↑ ↓ } P ≤ .05  
 ↓ ↓ }

TABLE 1

FLUID INTAKE, URINE VOLUME, CREATININE AND OSMOLAL CLEARANCES DURING STUDIES OF REPEATED DIVES TO 6.7 ATA.

SERIES A<sup>+</sup>

SERIES B<sup>+</sup>

PERIOD	†INT	EXC	CRTCLR	OSMCLR	PERIOD	†INT	EXC	CRTCLR	OSMCLR
CONTROL	2.140	1.525	72.5	2.46	CONTROL	2.203	1.412	110.6	2.31
DIVE #1	1.949	1.461	67.7	2.09	DIVE 1	1.207	1.152	94.4	1.93
2	2.049	1.127*	-	-					
3	2.449	1.251	62.3	2.29	DIVE 1	2.028	1.157*	124.9	2.18
Dive 1	2.537	2.029	72.2	2.92	DIVE 1	2.034	1.355	111.4	2.23
2	2.207	1.327	-	-					
3	2.203	1.386	80.5	2.55	DIVE 1	2.204	1.385	110.2	2.43
Dive 1	2.613*	1.736	77.6	2.31					
2	2.150	1.130*	-	-	DIVE 1	2.153	1.800	113.9	2.37
3	2.273	1.203*	81.6	2.09	2	1.951	1.493	-	-
Dive 1	2.189	1.377	77.0	2.26	3	1.821	1.418	120.3	2.33
2	2.264	1.337	-	-	4	1.877*	1.392	-	-
3	2.049	1.613	78.1	2.99	5-6 (av)	2.020	1.396	100.8	2.32
4	2.303	1.569	-	-	7-8 (av)	1.999	1.156	93.8	1.85
5-6 (av)	2.256	1.633	90.7	2.57	9-10 (av)	2.021	1.105*	-	-
7-8 (av)	2.296	1.390	82.2	2.53					
9-10 (av)	2.831	1.929	-	-					

+ Series A: 4 dives - 3 days apart

Series B: 5 dives - daily

† INT = Fluid intake, liters

EXC = Urine excreted, liters

CRTCLR = Creatinine clearance, ml/min

OSMCLR = Osmolal clearance, osmoles/min

\*Significant difference from control values by paired t-test. p ≤ 0.05

#Days post-dive

clearances resulted from the daily repeated dives.

Table 2 presents the results from analyses of cortisol levels in the serum of the subjects of series A. These data were obtained for evaluation of generalized stress reactions experienced during repeated diving. Values for the hormone are shown both for samples obtained at the standard blood collection time, 0730, and for experimental samples collected at 1300, one hour following the dives. Since the production of cortisol occurs on a cyclic basis throughout the 24-hour day, control data are included which show cortisol values for blood collected at 1300 hours four weeks following the experiment. While significant differences occur between the predive control samples and the one-hour post-dive samples, these can be accounted for by the changes in serum cortisol induced by the normal daily cyclic variations. All of the values collected at 0730 fall within a very narrow range while those obtained at 1300 are lower but also within equally confined limits.

#### DISCUSSION

Several questions continue to exist, concerning the role played by various pressurized gaseous environments in the alterations of tissue fluid status that accompany brief exposures to hyperbaric pressures. Diercks and Eisman (1977) have recently observed a transient hemodilution occurring after a series of daily repeated dives, and a brief period of hemodilution consisting of an 8% increase in plasma volume has been reported for the series A dives which are discussed here (Jacey, et al, 1977).

Although only changes signifi-

cant at  $<.05$  are depicted in figure 1, in general the concentrations of serum components decreased following the dives. An apparent anomalous situation such as that encountered one hour following the third dive in the A series seems to have resulted from an accumulation of pressure/decompression stresses which tend to be most severe at about this point in the sequence. The possibility that stresses were greater following this dive than for most of the others in the series has been substantiated by the concurrent observation that hemodilution, as measured by decreased hematocrit and increased whole blood and plasma water, peaked following this pressurization (Jacey, et al, 1977). It was also reported that hemodilution was much more marked at one day than at one hour following these dives. The concentrations of the serum components, particularly following the third dive in series A, supports earlier observations that the biologic responses to the imposed compression/decompression stress follow a pattern of immediate mild hemoconcentration (figure 1) with an ensuing slight hemodilution (Jacey, et al, 1977).

Another explanation for the biochemical changes occurring at the time of the second and third dives of series A is the stimulation of adaptive reactions in the subjects. Popular lore among diving personnel accepts the occurrence of diving adaptation. On the other hand, the minimal biochemical alterations occurring during these dives seem to agree with the performance data obtained which have been interpreted as indicating that there was no amelioration of the negative effects of elevated nitrogen pressures with successive exposures (Dr. George Moeller, personal communication).

Since all of the subjects of

TABLE 2

SERUM CORTISOL LEVELS ( $\mu\text{g}/\text{dl}$ ) FOLLOWING 4 DIVES TO 6.7 ATA 3-DAYS APART.  
 MEAN  $\pm$ SEM. N = 7 UNLESS OTHERWISE NOTED IN ( ).

## 0730 HOURS

CONTROL 17.52  $\pm$ .93 (21)1 DAY 17.45  $\pm$ 1.303 DAYS 18.82  $\pm$ 1.00

## POST-DIVE

1 DAY 18.46  $\pm$ 1.443 DAYS 17.25  $\pm$  .98

## POST-DIVE

1 DAY 18.10  $\pm$ 2.163 DAYS 16.93  $\pm$ 1.60

## POST-DIVE

1 DAY 15.03  $\pm$ 2.613 DAYS 19.62  $\pm$ 1.335 DAYS 18.86  $\pm$ 1.407 DAYS 16.87  $\pm$  .51

## POST-DIVE

## 1300 HOURS

CONTROL 12.55  $\pm$ 1.35 (5)1 HOUR 12.75  $\pm$ .98

## POST-DIVE

1 HOUR 11.99  $\pm$ 1.22

## POST-DIVE

1 HOUR 12.87  $\pm$ 1.69

## POST-DIVE

1 HOUR 12.62  $\pm$ 1.01

## POST-DIVE

## 1ST DIVE

## 2ND DIVE

## 3RD DIVE

## 4TH DIVE

NOTE: No post-dive value was significantly different from its appropriate control.

the series A dives were able to complete the experimental program without symptoms of dysbarism, it must be assumed that this group contained no unadaptable individuals or those for whom the decompression table was inadequate. In the series B study, however, the three subjects who experienced mild bends may belong to another category of individuals for which adaptation to this particular stress sequence is more difficult. It has been demonstrated in animals that certain members of apparently homogenous populations are capable of adapting to specific stresses while others are quite incapable of doing so (Tappan, 1971). Our experience indicates that some individuals are considerably more susceptible to the development of bends than others. Normal ranges of biological variability, of course, would lead to the prediction that a particular subject may be able to adapt under some but not all conditions of health or psychological stress. Note that the subjects adversely affected by the repeated dives were forced to drop from the series B experiment at about the time of the second dive, when the adaptive processes seem to be most actively stipulated -- or stressed.

Several general observations regarding the influence of repeated diving may be made in the light of the data of these and earlier studies. Single dives to 188 FSW in compressed air and lasting less than one hour have not been shown to result in metabolic alterations that shift serum or urine components out of clinically normal ranges (Heyder and Tappan, 1974). However, during the series A study, significant electrolyte and protein changes occurred indicating brief periods of both slight hemoconcentration and hemodilution with the

signs of hemoconcentration particularly evident at one hour after the third and fourth dives. Alterations in blood water content and most hematocrit changes for these dives reported by Jacey, et al (1977), seem to complement the picture of such cyclic blood volume fluctuations. It may also be noted that the tendency for immediate post-dive changes increases when two or three dives are carried out within the span of a few days. However, the changes do not progressively accumulate after the first few pressure exposures, and the serum components tend to return to control ranges between hyperbaric exposures if 72 hours are allowed to intervene between dives. On the other hand, daily dives result in increases in serum creatinine and albumin/globulin ratio, and decreases in phosphorus and osmolality for as long as three days following the fifth pressure exposure. Thus it appears that there is a delay in the subject's ability to completely recover during the period when they are exposed to daily pressurizations.

In spite of some hematologic and biochemical indications of physiologic stress accompanying short-term dives in air (Jacey, et al, 1977; Heyder and Tappan, 1973B), no strong evidence has become apparent during these experiments to contradict the working assumptions that such dives may be made on a daily basis. No dive-related increases in serum cortisol or urinary ketosteroid levels were detected nor were there increases in protein metabolite excretion that would indicate increased tissue catabolism. To the extent that serum uric acid levels reflect the degree of emotional stress perceived by the subjects (Clark, et al, 1975; Zir, et al, 1973), we do not conclude that the men were apprehensive about the

dives in which they were participating, although several relatively novice divers were included among the subjects.

Despite the lack of large biological alterations in divers subjected to repeated brief hyperbaric exposures, it must be remembered that three divers experienced symptoms of dysbarism during these experiments. It should be noted however, that no abnormal changes that might be predictive of dysbarism were observed in their serum or urine metabolites prior to the event. A case of documented decompression sickness resulting from an exposure of approximately 30 minutes at 188 FSW in compressed air has been reported from this laboratory (Jacey, et al, 1976). It is obvious that careful attention to details of allowable exposure times and proper decompression routines must be paid in order to perform short-term dives safely and uneventfully.

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19. KEY WORDS (Continue on reverse side if necessary and identify by block number)  repeated diving; compressed air diving; serum constituents (changes); urinary metabolites		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Eighteen qualified U. S. Navy divers underwent either of two series of dives to 6.7 ATA for 45-50 minutes in compressed air in a dry chamber: 4 dives at 3-day intervals or 5 dives performed daily. Serum and urinary components were monitored before and after the dives to compare the biologic influence of repeated diving with the known effects of hyperbaric saturation exposures. No diuresis or changes in glomerular filtration rate were demonstrated to result from the repeated sequences of pressurization and decompression. Biochemical evidence supports the previous finding of a hemodilution resulting		

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20. from repeated short-term pressure exposures. The increasing extent of metabolic changes during the first few dives leads to the conclusion that adaptation to repeated dives may begin to play a role after two or three dives particularly when performed at three-day intervals. While no cumulative effects beyond those associated with 2-3 dives are apparent when dives are performed with a few days intervening, limited biologic changes occur when dives are performed daily and seem to require an extended recovery period to allow metabolic processes to return to a state of predive equilibrium.

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