

BIOCHEMICAL RESPONSES OF MEN TO SIMULATED AIR DIVES  
OF 100 FEET

by

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## SUMMARY PAGE

### THE PROBLEM

To evaluate the effect of simulated shallow dives in an air environment on mineral and nitrogen metabolism, distribution, or loss, in men using standard Navy diving procedures.

### FINDINGS

The present findings support earlier observations that an extended period is required for biochemical equilibria to become re-established following exposure to pressure/decompression. Tentative evidence is provided here that recovery times of 7-9 days may follow shallow or medium depth dives.

### APPLICATIONS

Since certain potential effects of diving, such as dysbaric osteonecrosis, may be related to frequency and type of pressurizations, careful consideration should be given to providing sufficient recovery time between dives. Similarly, it is hoped that information will soon be available to estimate the time between each particular exposure which will best protect the long-term health of divers.

### ADMINISTRATIVE INFORMATION

This investigation was conducted as a part of the Bureau of Medicine and Surgery Research Work Unit MR041.06.01-0026BXKK - Biochemical Responses to Stresses of Submarine and Diving Environment. The present report is No. 2 on this Work Unit. The manuscript was approved for publication on 19 March 1974 and designated as Naval Submarine Medical Research Laboratory Report No. 774.

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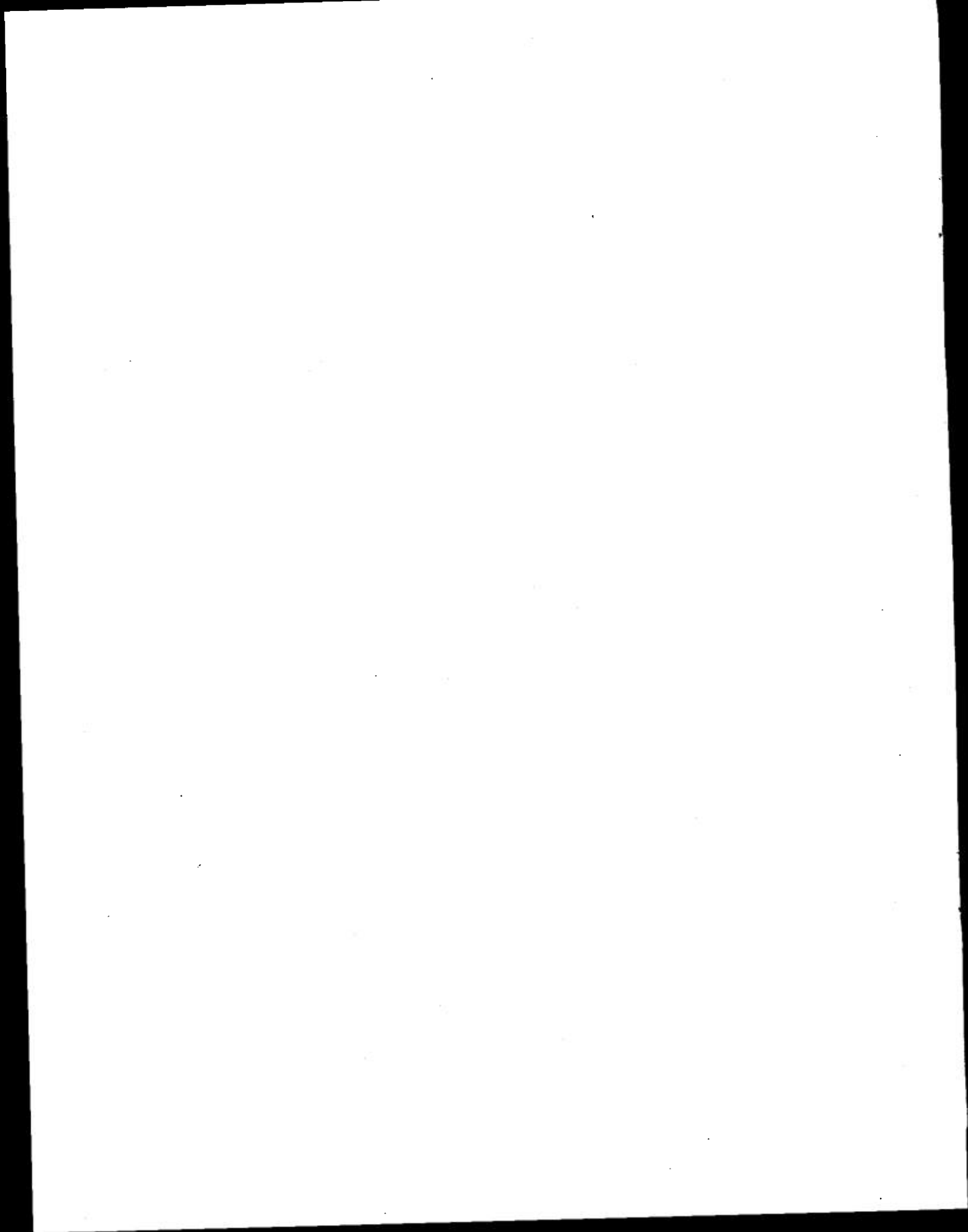
## ABSTRACT

Six men were subjected to a simulated dive in air to 100 FSW for one hour followed by a 70-minute decompression. Total 24-hour excretions of 12 urinary parameters were measured for 2 days prior to and 11 days following the dives. Blood serum constituents were determined in pre-dive samples, in samples taken immediately upon reaching the surface and in sera obtained for 9 post-dive days.

Urinary hydroxyproline increased on the 7th day while phosphorus decreased on the 6, 8, and 9th days post-dive. In addition, urinary solute decreased on the 6th day, and protein excretions decreased on both the 6th and the 8th day. The urinary Na/K ratios decreased during the 4-6th days post-dive.

Serum electrolyte imbalance occurred during the first 24 hours after the dive and again on the 7th day. The first episode results primarily from low potassium excretion while the second from a high sodium output. Peaks in serum phosphorus concentration on the 2nd and 5th post-dive day coincide with small increases in urinary phosphorus excretion.

The present findings provide tentative evidence that recovery periods of 7-9 days may necessarily follow shallow or medium depth dives.



# BIOCHEMICAL RESPONSES OF MEN TO SIMULATED AIR DIVES OF 100 FEET

## INTRODUCTION

Changes in the number of circulating thrombocytes result from a variety of conditions to which men or animals are exposed during actual or simulated diving operations.

Several reports have been made of the relationship of platelet clumping to decompression injury in experimental animals<sup>11,12</sup>. Bennet<sup>1</sup> and Bennett and Gray<sup>2</sup> as well as Philp et al.<sup>10</sup> have also described platelet decreases in human subjects following dives to 1500 ft. in helium-oxygen mixtures or to 300 ft. in air.

Martin and Nichols<sup>9</sup> first reported thrombocytopenia in man after an apparently uneventful dive to 100 ft. of sea water (FSW), breathing compressed air for 1 hour followed by a decompression of 122 minutes. Statistically significant decreases in platelet numbers were evident during the second and third day after decompression.

The present experiment was undertaken to investigate both the etiology of platelet decreases which accompany short exposures to shallow or moderate depths and to evaluate biochemical parameters which might shed light on associated physiological processes or mechanisms. A report on the hematologic studies performed for these dives has been presented separately<sup>16</sup>.

## MATERIALS AND METHODS

Six healthy male volunteers were subjected to hyperbaric exposures con-

sisting of simulated dives in air to 100 FSW for 60 minutes with decompression on a 100'/70 minute schedule<sup>15</sup>. Two subjects were studied in each of three dives.

On the two days preceding his dive, each man collected 24 hour acidified urine specimens. In addition, blood samples were withdrawn 1 day and 1-1/2 hours prior to the dives. The average results of the two samplings of blood and urine served as the control for each man.

Twenty-four-hour urine specimens were collected for 11 days following the dive with post-dive day 1 including the period of the dive. Blood was withdrawn immediately upon surfacing and at 24-hour intervals for 9 days thereafter.

During the studies the men refrained from eating ice-cream, jello, or soft candy to avoid excessive dietary intake of gelatin,—a rich source of hydroxyproline. The method of Hosley et al.<sup>7</sup> was used to determine the urinary hydroxyproline.

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. Serum chloride values were measured using a Buchler-Cotlove Chloridometer Model 42008.

Total serum and urinary calcium was 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, creatinine, and uric acid 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.

Urinary protein excretion was measured using the turbidometric method of Tappan<sup>13</sup> in an Aminco spectrophotofluorometer.

## RESULTS AND DISCUSSION

The data for the 24-hour excretions of twelve urinary parameters measured for the control days and for 11 days following the dives are shown in Table 1. Figure 1 (A, B) illustrates the concentrations of serum components for control samples, for serum samples obtained immediately upon completion of the dives and for sera representing 9 post-dive days.

Urinary hydroxyproline excretion was significantly higher than control levels at 7 days post-dive. Phosphorus, total osmoles, and total protein excretions were low on the sixth post-dive day and were related to a low urine output at this time. Significantly low excretions of phosphorus also occurred on the 8th and 9th days while protein output decreased on the 8th day. These latter changes were not directly correlated with low urine volumes. Such observations, together with the tendency for most of the excretion parameters to return toward normal levels during the second week of the study, suggest that

repair processes which are known to occur for at least 5 days after air dives<sup>6,8</sup> are essentially complete at 7 to 9 days post-dive. After this time the organism apparently has returned to the normal ranges of metabolic equilibria.

The sharp reactionary responses seen in other human or animal experiments on the first day after simulated air dives<sup>5,6</sup> seem to be expressed very much more mildly in this series of experiments. In fact, the responses observed here appear to be reflected primarily during the second to fourth days after the pressure episodes. These differences in response sequence may result from the relatively mild stress imposed and the short duration of exposure to pressure.

It may be reasonably speculated from our data, since both urinary hydroxyproline and phosphorus excretion may reflect bone metabolism, that bone responses occur for no less than 9 days after pressure-decompression experiences. This long recovery time may well be indirectly responsible for the osseous damage observed in divers after many years of repeated pressure and/or decompression insults, or in other workers exposed to hyperbaric environments, since 7-9 day rest periods do not generally occur during operational or commercial diving programs.

It is not possible to evaluate the effects of blood removal and reinfusion<sup>16</sup> when superimposed on the stress of the simulated diving procedures used in these studies. Such additional stressors cannot be overlooked, however, and they may be expected to have played at least a minor role in the recovery

Table I. Post-Dive Urinary Excretion by Men Subjected to a Simulated Dive to 100 FSW While Breathing Air. Twenty-Four Hour Samples. Mean  $\pm$  Standard Error of the Mean

	Vol ml	Hydroxyproline mg	Ca mg	P mg	Na mEq	K mEq	Na/K	OSM osmoles	Prot. mg	Urea N g	Uric Acid g	Creat. g
Control**												
$\bar{X}$	1194	24.35	144.5	1136	166	56.0	3.0	.899	24.3	12.91	.418	1.43
SEM	170	2.68	24.4	157	21	5.5		.065	3.5	2.28	.091	.28
1 Day												
$\bar{X}$	1377	23.93	178.2	1261	178	60.6	2.9	.989	21.8	12.68	.388	1.73
SEM	298	1.96	35.2	110	24	9.2		.081	6.6	.91	.063	.23
2 Days												
$\bar{X}$	1321	22.05	156.0	1167	187	55.7	3.4	.985	18.0	10.65	.372	1.65
SEM	261	1.66	19.6	144	19	2.6		.028	2.9	1.31	.094	.26
3 Days												
$\bar{X}$	1290	27.57	153.9	986	152	48.1	3.2	.831	26.7	13.52	.310	1.86
SEM	172	5.69	31.1	158	18	6.2		.090	5.4	2.31	.042	.24
4 Days												
$\bar{X}$	1192	24.52	109.8	1039	141	58.7	2.4	.837	23.0	12.75	.382	1.79
SEM	247	3.79	18.7	124	20	7.5		.061	3.2	1.21	.097	.10
					* $\Delta$							
5 Days												
$\bar{X}$	1322	26.33	140.6	1614	135	51.1	2.6	.798	21.2	11.37	.372	1.73
SEM	227	5.31	23.8	428	26	11.8		.098	6.6	1.34	.047	.14
					$\Delta$							
6 Days												
$\bar{X}$	863	21.58	135.2	794	124	46.4	2.7	.741	9.5	9.83	.258	1.58
SEM	128	4.37	23.0	37	18	2.5		.052	3.7	.86	.042	.08
				*				*	*			
7 Days												
$\bar{X}$	1745	33.77	150.3	958	205	50.6	4.1	.852	17.3	10.41	.407	1.68
SEM	329	5.05	22.0	55	22	6.3		.031	8.3	1.03	.063	.12
		*		$\Delta$								
8 Days												
$\bar{X}$	1371	30.43	122.8	782	171	53.0	3.2	.819	12.0	8.70	.307	1.39
SEM	329	10.18	20.6	151	22	7.8		.095	6.2	1.21	.056	.19
				*					* $\Delta$			
9 Days												
$\bar{X}$	1583	26.15	188.0	879	190	46.4	4.1	1.066	12.5	8.83	.305	1.30
SEM	410	3.96	41.1	150	17	5.8		.155	5.5	1.76	.040	.22
		$\Delta$		* $\Delta$		$\Delta$			$\Delta$			
10 Days												
$\bar{X}$	1287	23.82	136.7	945	162	50.0	3.2	.815	18.0	9.82	.362	1.66
SEM	494	3.65	25.1	140	27	6.4		.128	9.1	.44	.105	.14
11 Days												
$\bar{X}$	1286	26.30	121.0	903	139	44.0	3.2	.844	20.6	8.95	.334	1.32
SEM	287	5.21	21.4	203	28	9.5		.147	10.5	1.98	.100	.29

\*\*Averages of 2 control days for each man

\*Significantly different from controls at the 5% level or better by the paired t test.

$\Delta$  Significant differences from controls at level of 5% or better on the basis of concentration of excretion product

processes that we have discussed. Some repair or replacement of blood constituents could be expected after removal of 450 ml. of blood from each subject<sup>16</sup> followed by handling, chemical treatments and reinfusion. Thus, the responses which we have attributed to indications of alterations in bone metabolism may to some degree result from procedural techniques.

In addition to the inferences which may be made concerning the post-dive recovery of bone or other tissue groups, it is of interest to examine the data for information concerning the response of the men to the generalized stresses imposed<sup>14</sup>. The low urinary Na/K ratios observed during the 4th to 6th days after the dives indicate a peak of steroid activity at this time. Those ratios primarily reflect the tendency toward retention of sodium rather than excretion of potassium during this mid-phase of the recovery period. Assuming that the hormones reflected by the Na/K ratios are anabolic<sup>4</sup> in their actions, their increased presence during the 4th to 6th post-dive days may foreshadow the recovery climax during the 7th to 9th post-dive days as described earlier. Although steroid hormone excretion measurements were not performed for these experiments, primarily because of the minimal stresses imposed and the extensive analytical work required for the determinations, very crude approximations of steroid excretion rates may be obtained by employing our previously derived correlation equations which utilized excretion rates of potassium, urea nitrogen, creatinine and other urine components to estimate hormone excretion<sup>14</sup>. By such calculations, if the mean data for

the various excretory products are employed, the ketosteroid levels apparently peak on the 6th or 7th post-dive day depending upon which equation is used.

Besides oscillations in several of the serum constituents with peaks occurring at 2- to 4-day intervals, the most striking observation from the serum data (Figure 1) seems to be electrolyte imbalance that occurred during the first 24 hours after the dives. A significant lowering of serum potassium is evident immediately upon completion of the pressure exposure with an increase in serum sodium by the end of the first day after the dives. These responses are complemented by a peak of urinary potassium excretion during day 1. The second significant peak in serum sodium on day 7, corresponding to a chloride peak and preceded by a potassium peak, correlates with a rebound in the sodium/potassium excretory ratios which were previously described.

We are unable to determine whether the apparent period of hemodilution as indicated by the serum osmolality on the 4th post-dive day is directly related to the recovery process. Nevertheless, hemoconcentration following pressure exposures is an expected phenomenon<sup>8</sup> and overcorrection for displaced equilibria are common in biological systems.

The peaks of serum phosphorus concentrations on the 2nd and 5th post-dive days correspond to small increases in urine phosphorus levels at these periods. These peaks do not seem to be directly related to any major fluctuations in serum calcium or urine calcium and hydroxyproline and would seem to thereby reflect phenomena other than significant changes in bone metabolism.

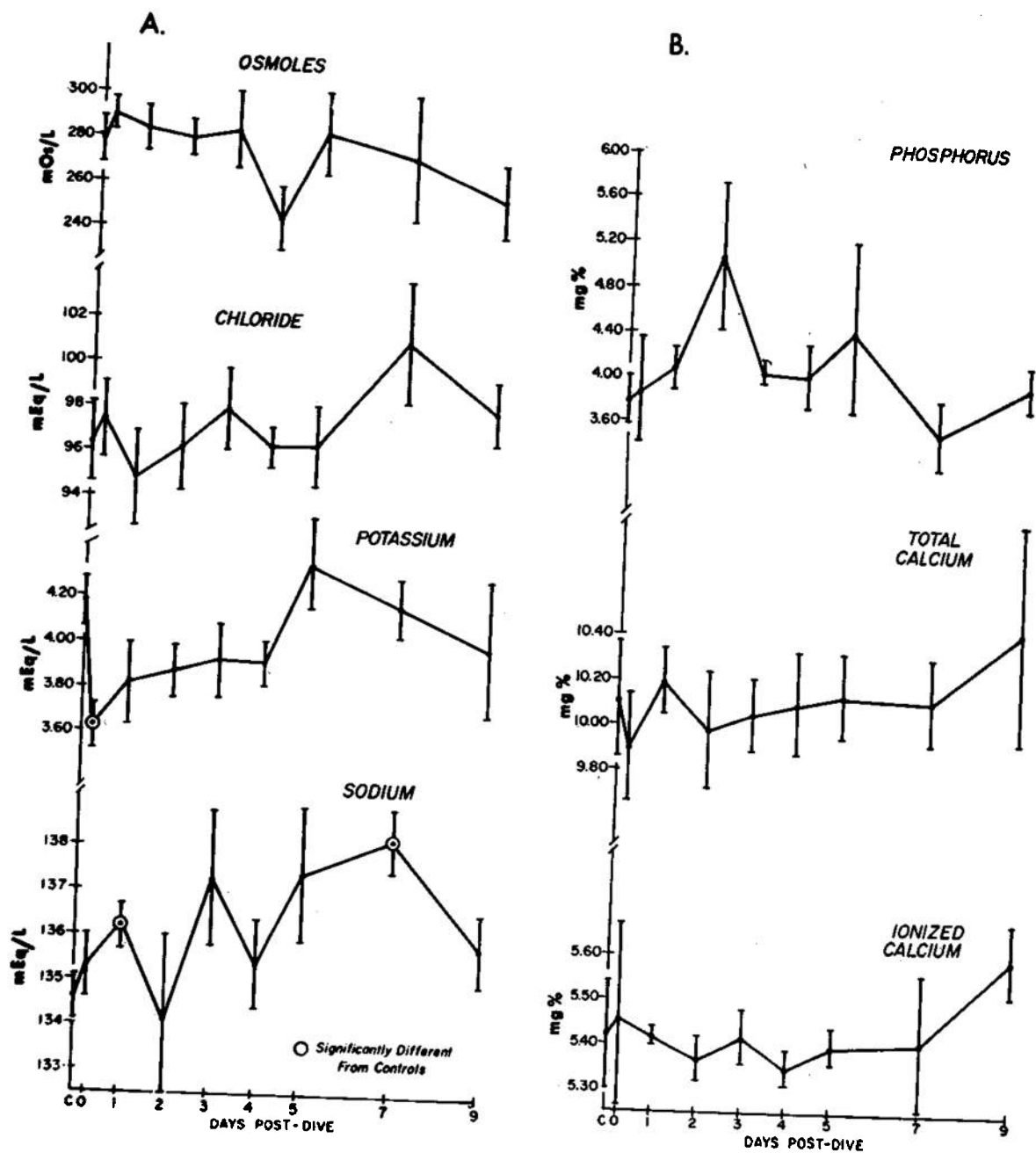
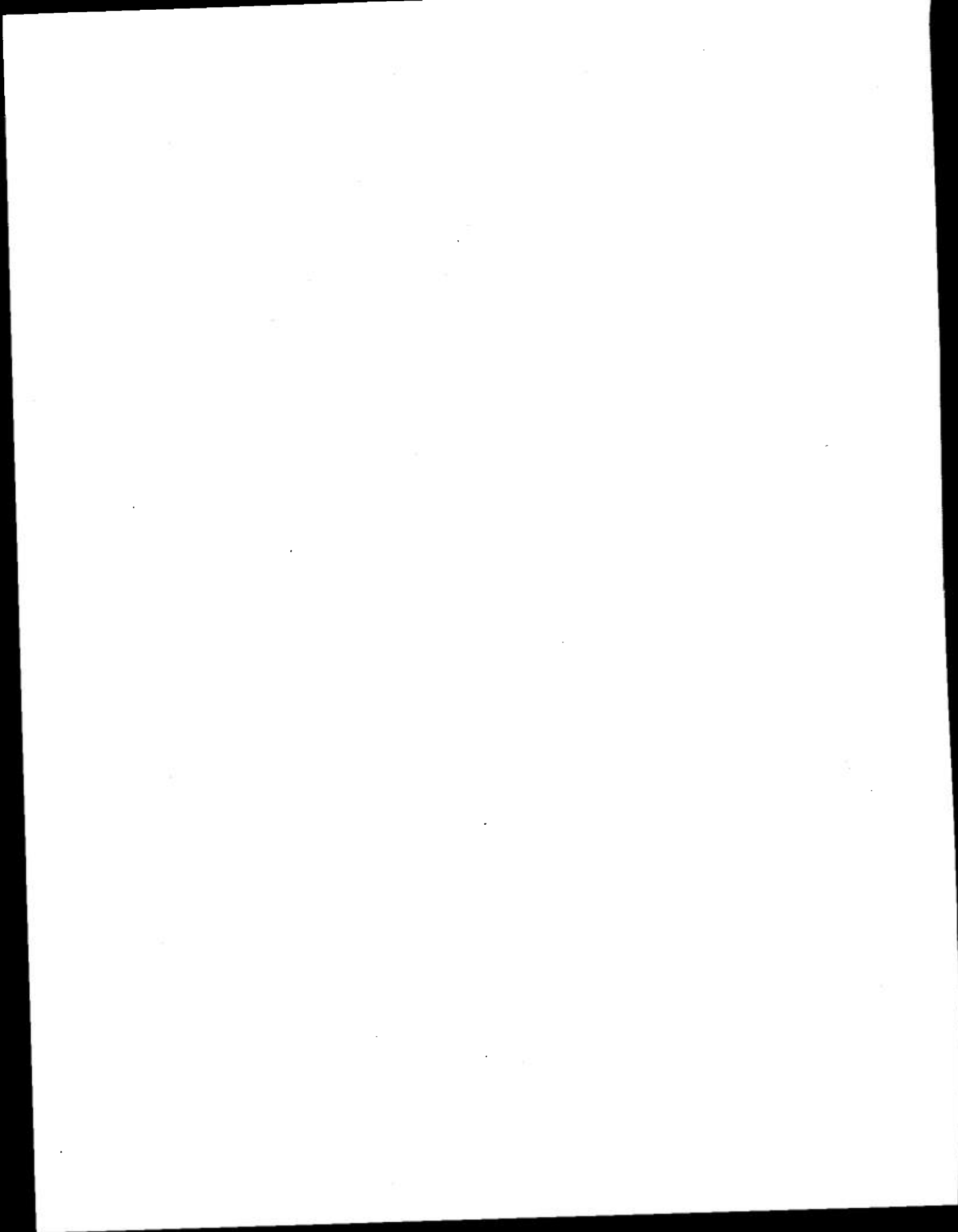


Fig. 1. Concentration of blood constituents in men exposed to simulated air dives of 100 FSW. Mean  $\pm$  standard error of the mean. N=6.

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