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ALTERATIONS IN THE METABOLIC AND SYMPATHETIC RESPONSE TO COLD EXPOSURE AFTER COLD AIR ACCLIMATION

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The experiments reported herein were conducted according to the principles set forth in the current edition of the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This technical report has been reviewed by the NMRI scientific and public affairs staff and is approved for publication. It is releasable to the National Technical Information Service where it will be available to the general public, including foreign nations.

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(19 cont'd) Body weight and basal metabolism did not change significantly over the eight week period. The oxygen consumed during the initial SCATT increased (Rest: 2.4 ml/kg/min; Cold: 3.6 ml/kg/min) significantly ($p < 0.001$) as did plasma norepinephrine (Rest: 385 pg/ml; Cold: 855 pg/ml). Plasma epinephrine did not change during the acute cold exposure. After the acclimation period, oxygen consumption was slightly reduced ($p < 0.09$), whereas plasma norepinephrine decreased significantly ($p < 0.01$) during the SCATT. There was a strong relationship ($R^2=0.44$; $p < 0.001$) between oxygen cost and plasma norepinephrine at rest and at the end of 30-minutes' cold exposure. Slopes and intercepts did not differ before ($Y = -433.2 + 304.8X$) or after ($Y = -412.8 + 308.8X$) the acclimation period. In conclusion, eighty cold exposures can reduce the metabolic and sympathetic response to an acute cold exposure without altering basal metabolism.

TABLE OF CONTENTS

	<u>Page No.</u>
ACKNOWLEDGEMENTS	iv
INTRODUCTION	1
METHODS AND MATERIALS	2
RESULTS	4
DISCUSSION	5
REFERENCES	7
FIGURE LEGENDS	9

APPENDIX

TABLE I	11
TABLE II	12
TABLE III	13
TABLE IV	14
TABLE V	15
FIGURE 1	16
FIGURE 2	17
FIGURE 3	18
FIGURE 4	19

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INTRODUCTION

The armed services, primarily the Navy and Coast Guard, are tasked with maintaining free passage and security in many geographical locations encompassing tropical and polar conditions. While personnel performance in heat has been well studied and ameliorative guidelines implemented, little is known about performance and survival in the cold. Acute cold exposure elicits frank shivering concomitant with a short-term elevation in basal metabolism. (1, 2, 3, 4) Extended cold exposure can produce mild to severe hypothermia resulting in reduced cognitive function and manual dexterity, thus, having a tremendous effect on mental and physical performance. Moreover, personal safety and health can be compromised in such environments.

The ability to adapt to these cold climates and possibly improve one's performance has been extensively investigated (5). There is strong evidence that localized cold adaptation can occur over many years of exposure (6). Whether this adaptation is due to local factors, central factors, or both is not clear. The mechanism responsible for whole body cold adaptation is not clearly understood, but it is generally believed that various types of whole body cold adaptation are possible (5). Serum triiodothyronine (T_3) is the primary hormone involved with many thermoregulatory and adaptive phenomena. As circulating T_3 levels appear to be decreased with prolonged Antarctic residence (7), we sought to determine whether T_3 supplementation and multiple cold air exposures would enhance human thermoregulation during a standardized cold air challenge. The objectives of the study were as follows:

PHASE I: Assess basal metabolic rate at rest before and after multiple cold exposures.

PHASE II: Assess the metabolic response during a cold (4° C) air challenge before and after multiple cold exposures.

METHODS AND MATERIALS

Subjects: Each subject provided informed consent. Subject characteristics are listed in Table I. Subjects were randomly assigned to a placebo or drug group. The control group (n=8) received placebo capsules filled with brown rice. The drug group (n=8) received capsules containing 30 ug triiodothyronine (T₃). Capsules were taken once a day in the presence of investigators throughout the eight week study.

Cold Acclimation protocol: Subjects were exposed to cold (4° C) air for 30 minutes, twice a day. The time between exposures was not less than one and one half hours (hr) and not greater than seven hr. This schedule was maintained for eight weeks beginning in January and ending in March with approximately 10 exposures per week for a total of 80 exposures. Body weight was obtained every two weeks during the course of the eight week study.

Basal metabolism protocol: Subjects arrived at the laboratory after an overnight fast and were instructed to lie supine in a reclining chair. They were covered with a blanket to keep them comfortably warm. After the subjects were in this position for 30 minutes, standard nose and mouth face masks (Sensormedics Inc., Anaheim, CA) were placed on them. Four 5-minute Douglas bags were collected and analyzed using oxygen and carbon dioxide analyzers from a Sensormedics 4400 metabolic cart. Bag volume (ATPS) was measured using a turbine system (Sensormedics Inc., Newport Beach, CA). Oxygen uptake and carbon dioxide output were determined from the expired gas fractions and ventilatory volume. Subjects repeated this procedure within 48 hours. At the

completion of the 80 exposures, the subjects repeated this procedure in the same manner.

Standard cold air tolerance test (SCATT): Approximately one week after basal metabolism measurements, subjects returned to the laboratory for the SCATT. Subjects arrived after an overnight fast and were seated in a wheel-chair. They remained in this position from 30 minutes prior to entry into the chamber throughout the cold exposure. An 18-G catheter was inserted into a brachial vein for blood sampling and kept patent with heparinized saline. Six minutes prior to entering the cold (4° C) chamber, two 3-minute Douglas bags and a 10-ml blood sample were obtained. Subjects were then wheeled into the cold chamber where they remained for 30 minutes. Six minutes prior to leaving the chamber, two 3-minute Douglas bags and 10-ml of blood were taken again. Subjects were then wheeled out of the chamber, and their recovery followed for an additional 30 minutes. Two 3-minute Douglas bags were obtained along with a 10-ml blood sample after 24 minutes of recovery. Bags were allowed to equilibrate to room temperature before determination of volume and expired gas fractions. Oxygen consumption (VO_2) and carbon dioxide output were determined as previously described.

Plasma for catecholamine analysis was separated by centrifugation at 3,000 rpm for 10 min at 4° C; 1-ml-aliquots were pipetted into tubes containing 6.5 mM glutathione and 8 mM ethylene glycol-bis-(B-aminoethyl)-N,N,N',N' - tetracetic acid (EGTA) and frozen at -70° C before analysis. Samples were extracted by alumina adsorption with 3,4-dihydroxybenzylamine (DHBA) included as an internal standard. Extracted samples were assayed by high pressure liquid chromatography using electro-chemical detection (Waters Division, Millipore, Milford, MA).

Statistical analysis: The effect of drug and cold exposure on dependent variables was analyzed by analysis of variance in a split-plot design. Significance was set at $p = 0.05$. All values are reported as mean \pm S.E.

RESULTS

Body weight: There were no significant [$F(4,15) = 1.75$; $p < 0.15$] changes in body weight over the course of the eight week study for either group.

Basal metabolic rate (BMR): Basal oxygen consumption was not significantly different between groups [$F(1,28) = 2.04$; $p < 0.16$] before or after the study (Fig. 1). Individual values are listed in Table II.

Standard cold air tolerance test (SCATT):

OXYGEN CONSUMPTION - The basal VO_2 prior to the acute cold exposure was not different between groups before or after the acclimation period. There was no overall group effect [$F(1,14) = 0.38$; $p < 0.54$] during the SCATT before or after the acclimation period. Oxygen consumption increased significantly [$F(2,56) = 58.56$; $p < 0.001$] in response to the cold (4° C) air challenge (Rest 2.4 ml/kg/min; Cold 3.6 ml/kg/min). After the acclimation period, there was a slight [$F(2,56) = 2.42$; $p < 0.09$], but not significant attenuation in the metabolic response to the SCATT (Fig. 2). Individual values are listed in Table III.

CATECHOLAMINES - Plasma epinephrine (EPI) did not change appreciably during the initial SCATT [$F(1,14) = 1.08$; $p < 0.35$] nor after the acclimation period [$F(2,52) = 1.13$; $p < 0.34$]. Individual plasma EPI values are listed in Table IV. Plasma norepinephrine (NE) increased significantly [$F(2,52) = 27.9$; $p < 0.001$] in response to the initial SCATT. This response was reduced [$F(2,52) = 6.5$; $p < 0.001$] after the acclimation period (Fig. 3; Table V).

METABOLIC AND SYMPATHETIC INTERACTION - There was a significant correlation ($R^2 = 0.44$; $p < 0.001$) between plasma norepinephrine and oxygen consumption (Fig. 4). Resting values are represented in the lower portion of the graph and demonstrate very little scatter. The response after the 30-minute cold exposure is represented in the upper portion and exhibits extreme scatter. The regression lines for January ($Y = -433.2 + 304.8X$) and March ($Y = -412.8 + 308.8X$) were not significantly different.

DISCUSSION

We present data demonstrating that after eighty 30-minute cold air exposures basal metabolism is unchanged in two groups. Modest increases in basal metabolism have been reported after both polar (7, 8, 9, 10, 11, 12) and less stringent environmental conditions (1). After a 10-day stay in cold (10° C) ambient conditions, soldiers experienced significant elevations in BMR (1). The soldiers sleeping in unheated huts with sleeping bags experienced a greater (16%) response than those sleeping in tents with sleeping bags (9%). In contrast, the changes in basal metabolism after cold water immersion studies are not as convincing (3, 4, 13, 14).

Prolonged exposure to cold environments without appropriate thermal protection can reduce core temperature and decrease manual dexterity. Moreover, mild to severe hypothermia has been reported to reduce cognitive ability (15). With such debilitating effects on mental and physical performance, a goal of cold acclimation would seem to be either the maintenance of normothermia or some adjustment in body temperature (e.g. lower core temperature), while maintaining cognitive function in either case. Milan et al. reported that after a 12-month Antarctic residence the response to a cold air test was significantly reduced (11). Personnel working outside and

under Arctic conditions had a lower metabolic response to a cold air test than personnel working inside (7). The results obtained from our cold air acclimation study are in general agreement with these previous studies. The reduction (15%) in the metabolic response to the SCATT after acclimation is consistent with the 17% attenuation in plasma norepinephrine. Our findings support previous work reporting a relationship between plasma norepinephrine and metabolism (16). Since both plasma NE and VO_2 are reduced after cold exposure in our study, it seems reasonable that the relationship between plasma NE and VO_2 after acclimation remains unchanged. Further investigation pertaining to the synergism between sympathetic activation and metabolic thermoregulation is necessary.

In conclusion, multiple cold air exposures reduce the metabolic response to a cold air test without affecting basal metabolism. Moreover, the sympathetic response to a cold air challenge is also attenuated. Repeated exposure to cold induces a reduction in circulating plasma norepinephrine and oxygen consumption, but does not alter their relationship. Triiodothyronine administration does not appear to influence human cold air acclimation.

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FIGURE LEGENDS

- Figure 1. Basal oxygen consumption before (January) and after cold acclimation (March). Mean \pm S.E. of LS mean.
- Figure 2. Metabolic response to an acute, 30-minute cold (4° C) air challenge for 16 men. Mean \pm S.E.
- Figure 3. The change in circulating plasma norepinephrine after an acute, 30-minute cold (4° C) air challenge in 16 men. Mean \pm S.E.
- Figure 4. The relationship between oxygen consumption and plasma norepinephrine at rest and after 30 minutes of cold (4° C) air exposure in 16 men. The figure depicts predicted line (Y = 176.5 (S.E. = 101.3) X - 2.44 (S.E. 30.5) with 95% confidence intervals. Equation was determined using pre and post data points acclimation.

APPENDIX

TABLE I. Descriptive characteristics

		<u>Drug</u>	<u>Placebo</u>
AGE	MEAN	29.3	30.9
	S.D.	1.2	3.8
HT	(cm)	178.2	180.6
		6.0	2.9
WT	(kg)	83.5	83.8
		10.2	7.4
PERCENT FAT	(%)	11.6	13.2
		4.6	1.8
BODY SURFACE AREA	(m ²)	2.01	2.03
		0.12	0.10

TABLE II. Individual basal metabolism values (ml oxygen/ min/ kg obtained on two consecutive days before (J) and after (M) cold acclimation period.

S		Day 1				Day 2			
		Bag 1	Bag 2	Bag 3	Bag 4	Bag 1	Bag 2	Bag 3	Bag 4
1	J	2.82	3.20	3.05	3.08	2.98	3.08	3.14	3.26
	M	3.48	3.17	3.22	3.28	3.41	3.41	3.15	3.04
2	J	3.13	2.98	2.93	2.89	2.72	2.66	2.67	2.65
	M	2.52	2.65	2.72	2.54	2.52	2.72	2.92	2.57
3	J	2.39	2.69	2.54	2.49	2.56	2.41	2.54	2.61
	M	2.73	2.58	2.74	2.81	2.84	2.87	2.85	2.95
4	J	2.78	2.98	3.26	2.88	2.61	2.43	2.78	2.67
	M	2.54	3.17	2.78	3.16	2.49	2.18	2.37	2.45
5	J	2.23	2.30	2.26	2.29	2.12	2.12	2.22	2.20
	M	2.42	2.28	2.29	2.39	3.03	3.24	3.09	3.07
6	J	2.42	2.52	2.40	2.48	1.51	1.63	1.71	2.05
	M	2.72	2.86	2.93	3.11	2.78	2.73	2.39	2.73
7	J	3.11	2.98	2.89	2.84	2.90	2.42	2.84	2.55
	M	2.55	2.45	2.84	2.95	3.27	2.54	2.69	2.29
8	J	2.13	2.05	2.15	2.12	2.14	2.28	2.35	2.27
	M	3.11	3.25	3.47	3.31	3.05	2.98	2.92	2.98
9	J	2.84	2.82	2.85	2.94	3.02	2.81	2.82	2.76
	M	2.63	2.64	2.72	2.68	2.60	2.85	2.39	2.43
10	J	3.40	3.27	3.18	3.12	2.93	3.16	2.97	3.13
	M	2.86	2.64	2.78	2.93	2.78	3.01	2.64	2.89
11	J	2.40	2.35	2.44	2.31	2.31	2.25	2.26	2.24
	M	2.19	2.34	2.57	2.03	2.33	2.44	2.17	2.33
12	J	3.07	2.90	3.38	3.21	3.37	3.04	3.42	4.26
	M	2.92	3.21	3.05	3.13	3.19	3.52	3.29	3.21
13	J	3.54	3.68	3.67	3.77	2.96	3.03	3.04	2.96
	M	2.93	2.77	2.55	2.74	2.74	2.50	2.98	2.63
14	J	2.15	2.18	2.13	2.11	2.68	2.74	2.77	2.64
	M	3.26	3.27	2.97	2.84	2.73	2.80	2.76	2.54
15	J	3.01	2.80	3.13	2.81	2.37	2.33	2.38	2.34
	M	2.78	2.91	2.55	2.70	1.97	1.95	2.14	2.22
16	J	2.70	2.80	2.31	3.33	1.78	1.59	2.12	2.08
	M	2.81	2.78	2.67	3.05	2.78	2.66	3.28	2.82

TABLE III. Individual oxygen consumption values (Douglas bags- B) at rest, after 30 minutes' cold and after 30 minutes' recovery. Values expressed as ml oxygen/min /kg.

S		Rest		Cold		Recovery	
		<u>B 1</u>	<u>B 2</u>	<u>B 1</u>	<u>B 2</u>	<u>B 1</u>	<u>B 2</u>
1	J	2.78	3.36	4.15	4.38	3.94	3.20
	M	3.30	2.76	3.76	3.28	3.08	2.83
2	J	2.92	2.80	3.26	3.02	2.84	2.49
	M	3.14	2.66	3.10	3.64	2.78	2.78
3	J	2.59	2.99	3.82	3.67	3.10	3.10
	M	2.87	2.80	3.58	4.33	4.00	3.64
4	J	3.01	3.05	3.05	4.06	2.46	2.68
	M	2.57	2.16	3.37	3.39	3.22	2.78
5	J	2.14	2.08	4.09	4.00	2.95	2.46
	M	2.14	2.81	3.72	3.33	2.31	2.57
6	J	2.86	2.86	4.00	4.14	3.20	2.64
	M	2.18	2.29	4.05	4.05	2.82	2.95
7	J	3.05	2.44	4.26	4.44	3.20	3.94
	M	2.64	2.34	4.18	2.85	3.15	2.42
8	J	3.03	2.88	4.61	4.85	2.44	2.74
	M	2.39	2.69	4.08	3.76	2.98	3.78
9	J	2.50	2.28	3.66	2.42	2.82	2.27
	M	2.40	2.56	3.38	3.67	3.04	2.23
10	J	2.79	2.94	5.00	5.54	3.47	3.13
	M	2.88	2.68	3.71	3.71	3.16	2.85
11	J	2.33	2.23	4.60	4.48	2.34	2.61
	M	2.19	2.47	3.97	3.78	2.50	2.39
12	J	2.96	2.95	3.49	4.29	3.19	2.86
	M	2.80	2.56	3.05	2.75	2.87	2.85
13	J	3.30	2.76	3.98	3.78	3.13	2.86
	M	2.76	2.80	3.81	4.12	2.80	2.31
14	J	2.67	3.01	3.01	3.28	2.99	2.92
	M	2.46	2.59	4.37	4.56	3.26	3.26
15	J	2.94	2.78	8.41	7.28	3.95	4.27
	M	2.52	2.66	4.49	4.68	3.35	3.37
16	J	2.56	2.40	4.87	4.87	2.95	3.59
	M	3.15	2.38	2.91	2.88	2.84	3.26

TABLE IV. Individual plasma epinephrine (ng/ml) during a standardized cold (4° C) air challenge before (J) and after (M) cold acclimation.

S		Rest		Cold		Recovery
		<u>-30"</u>	<u>0"</u>	<u>15"</u>	<u>30"</u>	<u>30"</u>
1	J	45	83	40	64	61
	M	62	56	40	58	63
2	J	26	38	56	68	29
	M	49	82	34	38	61
3	J	42	23	53	78	41
	M	31	21	57	50	25
4	J	76	105	70	110	59
	M	102	89	52	65	64
5	J	27	30	43	55	55
	M	43	28	75	47	50
6	J	45	61	63	105	55
	M	54	38	58	101	33
7	J	45	20	20	51	54
	M	58	82	79	57	77
8	J	63	45	39	46	35
	M	44	20	34	25	28
9	J	97	111	51	74	83
	M	36	41	43	83	63
10	J	55	20	20	20	20
	M	35	35	20	20	20
11	J	46	37	39	37	31
	M	20	23	20	20	20
12	J	160	117	125	22	84
	M	133	160	401	465	83
13	J	86	94	47	66	66
	M	115	95	41	57	76
14	J	91	38	20	29	28
	M	59	48	25	27	24
15	J	24	40	27	20	43
	M	30	41	45	23	56
16	J	71	49	20	20	20
	M	20	20	20	20	20

TABLE V. Individual plasma norepinephrine (pg/ml) during a standardized cold (4° C) air challenge before (J) and after (M) cold acclimation.

S		Rest		Cold		Recovery
		-30"	0"	15"	30"	30"
1	J	148	136	1030	1228	579
	M	237	352	1148	1444	1488
2	J	497	364	776	658	532
	M	427	498	871	954	750
3	J	309	268	1085	1255	704
	M	316	224	444	646	636
4	J	252	237	850	980	457
	M	251	280	564	488	362
5	J	545	491	784	934	491
	M	487	429	509	493	409
6	J	302	226	652	707	431
	M	427	273	422	659	455
7	J	208	281	841	897	581
	M	335	274	260	233	294
8	J	246	352	490	498	602
	M	232	194	327	475	689
9	J	741	640	1440	801	573
	M	424	899	714	569	243
10	J	214	448	635	805	629
	M	345	421	694	1064	648
11	J	180	228	436	588	376
	M	296	391	380	631	574
12	J	405	321	586	1245	434
	M	341	581	724	498	229
13	J	334	249	1092	1202	464
	M	361	258	861	949	424
14	J	306	222	810	1122	678
	M	285	247	770	1078	655
15	J	351	361	979	893	564
	M	326	364	662	693	438
16	J	566	438	407	527	559
	M	679	477	706	1002	670

FIGURE 1

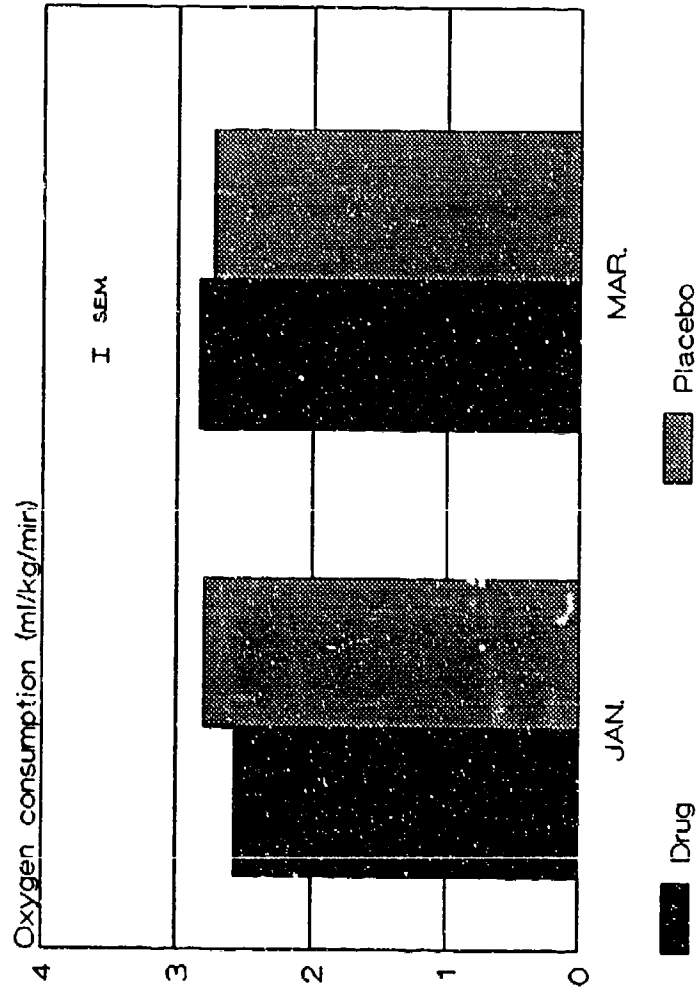


FIGURE 2

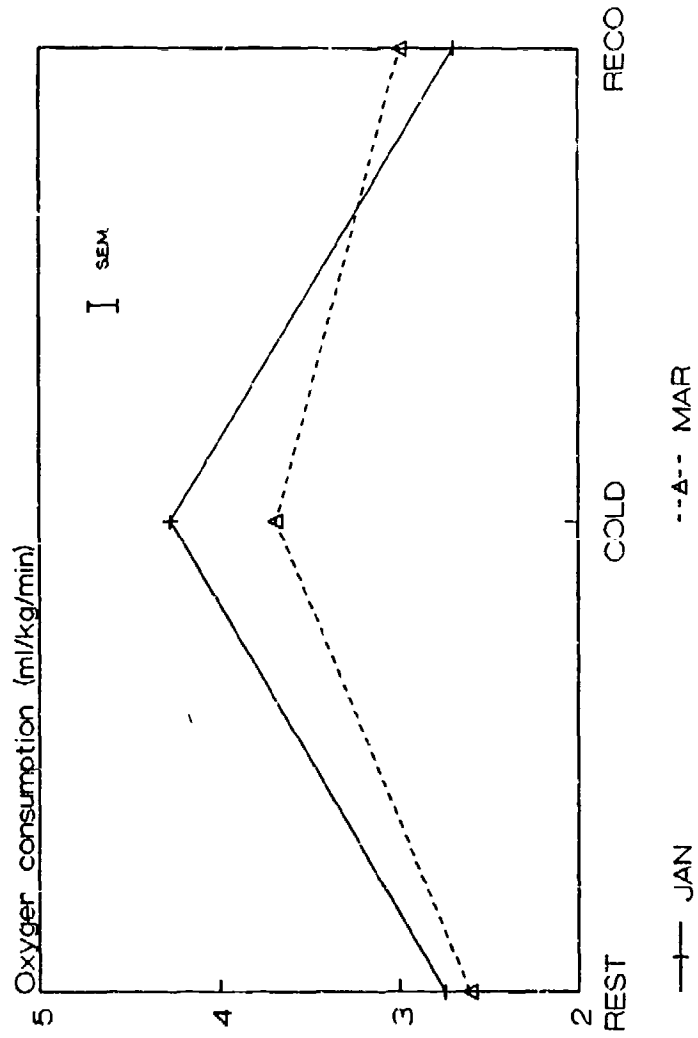


FIGURE 3

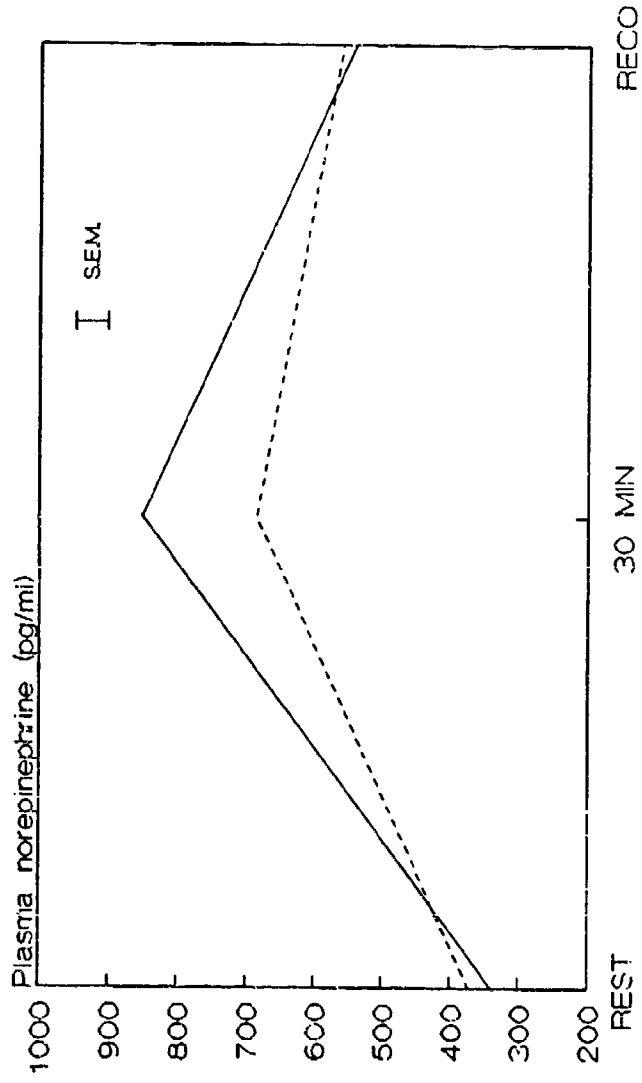


FIGURE 4

