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THE EFFECT OF NAP-OF-THE-EARTH (NOE) HELICOPTER FLYING ON PILOT BLOOD AND URINE BIOCHEMICALS

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Selected blood and urine chemistries were compared in helicopter pilots flying alternately nap-of-the-earth (NOE) and routine flight profiles. The NOE flights resulted in significantly higher urinary catecholamine excretion ( $P < .05$ ), serum uric acid ( $P < .05$ ) and blood lactic acid ( $P < .01$ ). Pre-flight cortisol was significantly higher than post-flight ( $P < .01$ ), and post-flight catecholamine excretion rate was higher than during the three hour post-flight sample period ( $P < .01$ ). The biochemical results are		

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consistent with the reports that NOE flight is physically more demanding in terms of muscular strain. The increased catecholamine excretion may indicate the perception of NOE flight as a more demanding and stressful activity than flight at higher altitudes. In light of previous work, the higher serum uric acid levels prior to NOE flight may provide a measure of the pilot's psychological preparation and possible performance during NOE flight.

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## THE EFFECT OF NAP-OF-THE-EARTH (NOE) HELICOPTER FLYING ON PILOT BLOOD AND URINE BIOCHEMICALS

### INTRODUCTION

Helicopter pilots have reported that low level and nap-of-the-earth (NOE) flights are substantially more stressful and fatiguing than normal higher altitude flight. This study in its entirety was conducted to test these claims and define changes in a pilot's physiology, biochemistry and performance characteristics as a result of NOE flight. In this portion of the project, various biochemical measurements were made on blood and urine specimens collected from helicopter pilots. On separate days the pilots were required to fly NOE and normal flight profiles. Differences between the two types of flying were determined and compared statistically. Performance differences have been reported in a previous publication.<sup>4,7</sup>

A number of blood and urine components have been shown to change in concentration or in excretion rate during various types of stresses (i.e., emotional stress, physical exhaustion, muscular exercise, acceleration, and combat fatigue). Probably the most extensively studied has been the 17-hydrocorticosteroid (17-OHCS) secretion by the adrenal cortex.<sup>1,10,11,12,18,20,21,23,24,39,40,41</sup> Increase in the level of 17-OHCS during flying is affected by the type of flying mission, level of the individual's responsibility, duration of the flight, and the time of the flight with respect to the normal sleep cycle.

The catecholamines, epinephrine and norepinephrine, have also been studied in pilots, air traffic controllers, and other volunteers subjected to stressful situations.<sup>6,10,11,16,20,21</sup> Plasma levels of catecholamines are increased during race car driving.<sup>4,3</sup> Norepinephrine is positively correlated with improvement in performance during stress<sup>7,8</sup> and negatively correlated with fatigue ratings.<sup>8</sup>

Serum uric acid and cholesterol levels have also been studied in humans under stress.<sup>2,15,29,30,34,37,40,46</sup> Concentrations of these biochemicals appear to have some relation to the psyche. Rahe et al.<sup>35</sup> have found negative correlations between blood cholesterol and feelings of motivation, arousal and happiness, and positive correlations with a mood of depression, anger, fear or lethargy. Serum urate is elevated during periods of intense psychological stress.<sup>33</sup> It appears that those individuals striving against odds show elevated uric acid.<sup>37</sup> Serum urate is also correlated with highly motivated, achievement-oriented personalities.<sup>25,36</sup>

L-lactic acid is the glycolytic end product of carbohydrate metabolism in muscle, and it has been known for many years that the lactate

concentrate in blood increases after muscular exercise.<sup>14</sup> Pitts has also published data which suggests that lactic acid increases during anxiety in susceptible individuals.<sup>31,32</sup>

Serum creatine kinase (CPK), as well as other enzymes, has been shown to increase under certain conditions of muscular activity.<sup>9, 38,42,44</sup> Nuttall and Jones found that physical conditioning reduced or eliminated the increase in serum CPK and serum glutamate-oxalacetic transaminase (SGOT) after exercise.<sup>27</sup> In addition, Martin and Nichols found an increased serum CPK in divers undergoing rigorous training.<sup>19</sup>

#### MATERIALS AND METHODS

Subjects. The research subjects included six volunteer rotary wing Army flight instructors who were currently involved in the instruction of NOE procedures in the initial entry helicopter training program at Fort Rucker, Alabama.

Flight Profiles. The experiment was designed to test whether the blood and urine biochemicals of pilots flying NOE missions were different than when the same pilots were flying "normal" or routine missions at 1,000 feet above ground level (AGL). The NOE flight was conducted approximately 40-50 feet AGL and at 45 knots airspeed down a winding riverbed. This altitude was at or below treetop level. The course was approximately seven minutes in duration and was repeated three consecutive times. The flight from the heliport to the NOE course and back was flown at low level approximately 10 feet above treetop level. Total flight time was approximately 30 minutes. The "normal" or routine flight was conducted at 1,000 feet AGL and 80 knots airspeed for the same total time (30 minutes) as the NOE flight.

Each pilot flew both the NOE and the "normal" flight on separate days. Half of the subjects entered the study with NOE flight and half entered with the "normal" flight to balance any possible effects of repeated venipuncture. All flights were conducted in the forenoon.

Sampling. Blood samples were taken prior to and immediately following the completion of the flight. Subjects voided urine immediately before and immediately after flight. A third urine specimen was collected three hours after the flight. The following blood and urine components were measured. Blood lactic acid was measured by the method of Marback and Weil.<sup>17</sup> Immediately after sampling by venipuncture, 2 milliliters (ml) of blood was pipetted into 4.0 ml of ice cold, 8% perchloric acid and shaken vigorously for 30 seconds. The sample remained on ice for approximately three hours until returned to the laboratory for analysis. The precipitate was separated by centrifuging at 1500 x g (gravity) in a refrigerated Lourdes beta-fuge. The clear supernate was collected and analyzed for lactic acid by the Sigma Chemical procedure no. 826-UV.



Serum uric acid, creatine kinase (CPK), lactate dehydrogenase (LDH), plasma cholesterol, and glucose were measured by the Biodynamic Unitest System (Biodynamics, Inc., 9115 Hague Road, Indianapolis, Indiana 46250). Control samples were utilized before and after study sample analysis to insure accuracy.

Plasma cortisol was determined by the protein binding method of Newsome et al.<sup>26</sup> Steroids were extracted from the plasma with methylene chloride followed by chromatography on Sephadex LH-20 to separate cortisol. Recovery of cortisol was determined by the use of a low activity <sup>3</sup>H-cortisol [.1400 counts per minute (cpm)] internal standard added to the original plasma sample. An aliquot of the cortisol fraction was counted to determine percent recovery. Another aliquot of the cortisol fraction was combined with a two percent solution of corticoid binding globulin and high activity <sup>3</sup>H-corticosterone (approximately 220,000 cpm). The cortisol preferentially binds to the globulin displacing the <sup>3</sup>H-corticosterone. The solution was then counted on a Beckman LS-200B liquid scintillation counter after removal of the unbound <sup>3</sup>H-corticosterone by the addition of florisol. The radioactivity of the remaining solution was inversely proportional to the concentration of cortisol in the sample.

The urine specimens were acidified to pH 2.5 with 6N HCl and frozen until analysis. Analysis was completed within 45 days of sampling. Catecholamines were determined by a modification of the fluorometric procedure of Merrills.<sup>22</sup> Norepinephrine was used as a standard. Catecholamines were oxidized with potassium ferricyanide. Fluorescent trihydroxyindole derivatives were formed by the addition of alkali and were stabilized with ascorbic acid. Fluorescence was measured on an Amino-Bowman Spectrophotofluorometer.

#### RESULTS AND DISCUSSION

Nap-of-the-earth (NOE) type flying has a number of stressors that are not normally found to the same extent as with flight at higher altitudes. These stressors include: (1) An increased performance stressor is expected due to the exacting and precise performance that is required under NOE conditions. This type of performance would likely lead more rapidly to mental and muscular fatigue. (2) There is increased physical danger in NOE type flying. An engine failure at low altitude and airspeed is recognized as decreasing the choice of forced landing areas or the time available to accomplish aircraft recovery. This may cause an added conscious or subconscious stress on the pilot. (3) The stressor of competition may also exert an effect. The pilots in this particular experiment knew that their performance was being monitored and compared to other subjects.

Responses used as indices of stress in other studies have included a large number of physiological functions. In this experiment certain blood and urine components have been measured which have been demonstrated in previous studies to respond to various types of stressors. The level of these blood and urine constituents was evaluated to possibly provide insight into the differing degrees of stress experienced by pilots under the two types of flight conditions studied.

Table I shows the tabulation of mean values, standard errors and a statistical summary of all analyses. For easier visualization, the data is also shown graphically in Figures 1, 2, 3 and 4.

Cortisol and Catecholamine Evaluation. Figure 1 shows the results of the cortisol and catecholamine measurements. The stimulation of adrenocortical activity and hence the secretion of cortisol by the adrenal have been previously demonstrated in pilots and in individuals subjected to various types of stressors.<sup>1,10,11,12,21,23,24,39,40</sup> The mean values obtained during NOE flight were higher for both pre- and post-flight samples but the differences were not statistically significant. Preflight samples were, however, statistically higher ( $P < .01$ ) than post-flight, 14.4 versus 9.0  $\mu\text{g/dl}$  for local area or routine flight and 15.4 versus 11.8  $\mu\text{g/dl}$  for NOE flight. The normal plasma cortisol concentration is approximately 12  $\mu\text{g/dl}$ . These results could be interpreted as indicating anticipation of flying produced a greater cortisol response than the actual flight itself. The factors possibly contributing to the pilot's apparent anticipatory response would be the knowledge that his flying skills were being monitored or perhaps a subconscious reluctance or concern about the impending venipuncture. It is also possible the differences are the result of the natural diurnal pattern of plasma cortisol changes since the sampling period is during the downslope of the normal circadian pattern.<sup>28</sup>

The NOE type flying resulted in a higher exertion of catecholamines both immediately and three hours post-flight ( $P < .05$ ). The post-flight sample was also significantly higher than the three hour post-flight sample ( $P < .01$ ). The measurement of total catecholamines includes both epinephrine and norepinephrine. Therefore, the increases could represent an adrenomedullary response or a sympathetic nervous system response, or a combination of both. The response was similar to that reported in other volunteers subjected to other types of stressors.<sup>6,7,8,16,21,43,45</sup> The catecholamine results are consistent with the premise that NOE is a more stressful type of flying than missions flown at 1,000 AGL for the same time period.

The post-flight sample was significantly higher than the three hour post-flight sample. Due to the method of urine sampling, the post-flight sample represents both preflight excretion as well as excretion during the flight. In order to determine more precisely which period was contributing to the increase in catecholamines, urine samples were

TABLE I

Concentrations of selected blood and urine biochemicals in pilots flying alternately nap-of-the-earth (NOE) and routine or "normal" flight profiles. Differences were analyzed for significance by analysis of variance with a 2 x 2 factorial design. The F values and level of significance are shown opposite the concentration of each blood or urine component. NS means P > .10.

SAMPLE PERIOD	TYPE OF FLIGHT						F STATISTIC	
	"Normal" Flight		N.O.E. Flight		F	P	Pre vs. Post	Interaction
	Preflight	Postflight	Preflight	Postflight				
<b>BLOOD</b>								
Plasma Cortisol ( $\mu$ g/100 ml)	$\bar{X}$	14.42	9.00	15.42	11.83	2.2	12.0	0.5
	S.E.M.	2.33	1.65	3.34	2.78	NS	P < .01	NS
Lactic Acid (mg/100 ml)	$\bar{X}$	6.55	5.45	9.60	10.13	22.9	0.1	1.0
	S.E.M.	1.47	1.12	0.56	0.70	P < .01	NS	NS
Plasma Cholesterol (mg/100 ml)	$\bar{X}$	212.00	219.00	205.00	222.00	0.1	4.3	0.7
	S.E.M.	14.00	14.00	11.00	9.00	NS	P < .10	NS
Serum Uric Acid (mg/100 ml)	$\bar{X}$	6.68	6.93	7.80	7.68	6.1	0	0.2
	S.E.M.	.37	.39	.54	.54	P < .05	NS	NS
Serum CPK (I.U.)	$\bar{X}$	35.2	37.8	55.6	56.2	3.4	0	0
	S.E.M.	4.0	3.9	16.2	17.9	P < .10	NS	NS
Serum LDH (Wacker Units)	$\bar{X}$	87.9	105.7	117.8	116.2	4.1	0.7	1.0
	S.E.M.	5.9	16.2	7.8	9.6	P < .10	NS	NS
Plasma Glucose (mg/100 ml)	$\bar{X}$	76.3	81.2	68.2	78.4	1.6	3.1	0.4
	S.E.M.	10.3	4.3	6.5	7.8	NS	NS	NS
<b>URINE</b>								
Sample Period Urinary Catecholamines ( $\mu$ g/hour)	$\bar{X}$	4.86	2.50	8.95	3.81	4.9	10.1	1.5
	S.E.M.	0.80	0.26	1.94	0.70	P < .05	P < .01	NS
			3 hr. Postflight	Immediate Preflight	3 hr. Postflight	"Normal" vs. N.O.E.	Post vs. 3 hr. Post	Interaction

S.E.M. = standard error of the mean

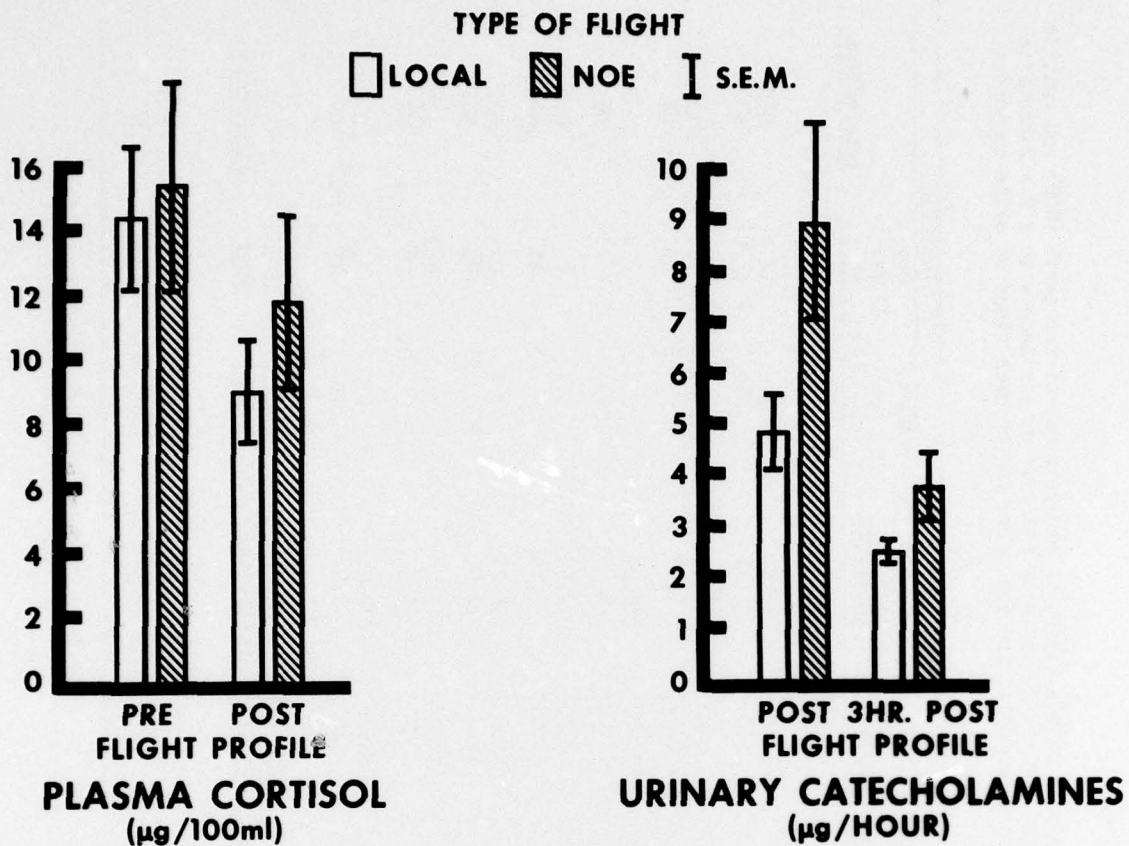


Figure 1. Plasma cortisol and urinary catecholamine response to NOE flight. Open bars: "normal" or routine flight at 1,000 feet AGL. Shaded bars: nap-of-the-earth flight at a below treetop level. SEM: Standard Error of the Mean is indicated at the top of each bar.

taken from another group of six pilots who flew the same NOE course at night. The samples were, however, taken immediately before the flight, representing a one-hour preflight period, and again after the completion of the flight. The results in Figure 2 show that the mean preflight excretion was slightly higher but not significantly different from the post-flight sample. Based on these results, it appears that the high catecholamine level of the post-flight sample that was found in the first experiment was probably the result of higher excretion both during the anticipatory preflight phase as well as the actual flight. The lower excretion rate found in the night study is most probably related to diurnal variation of catecholamine excretion. The low point of norepinephrine and epinephrine excretion is during the evening.<sup>5</sup>

Lactate, LDH, CPK Evaluation. Figure 3 shows the results of blood lactate, serum lactate dehydrogenase (LDH) and creatine kinase (CPK) analyses. All lactic acid means were within the normal range; however, it was found that the concentration of lactic acid was significantly higher ( $P < .01$ ) in blood samples taken during the NOE flights. The difference is greatest in the post-flight sample. The effect of muscular exertion to increase the concentration of blood lactate has been known for many years.<sup>14</sup> The doubling of blood lactate in the sample immediately following the NOE flight is considered as reflecting an increased muscular exertion and muscle tension during the NOE flight profile. Instructor pilots have noted that during NOE type missions, many pilots grasp the collective stick much more tightly and are, in general, more tense than at higher altitude flights. The higher lactate values measured in the preflight sample are more difficult to explain as there should have been no significant difference in the amount of muscular activity before any of the flights. The difference could be explained by differences in the catecholamine levels which are known to cause increases in blood lactate. In this study it was found that the two individuals with the greatest difference in preflight lactate levels also had the greatest difference in catecholamine excretion as measured in the immediate post-flight urine sample.

Serum LDH and CPK were not substantially affected by the type of flying but tended to be higher in the NOE samples ( $P < .10$ ). LDH values were somewhat above the normal range of 45-90 Wacker Units. The variation of the CPK values during the NOE flights was much higher than during "normal" flying. An increased variation resulting from stressful stimuli is not atypical for CPK. Martin and Nichols have shown that a proportion of individuals in their study were particularly labile to elevations in CPK levels during periods of diving training.<sup>19</sup> The period of increased muscular exertion during NOE was probably not long enough, however, to elicit a statistically significant response in LDH and CPK levels.<sup>9</sup>

### NIGHT NOE

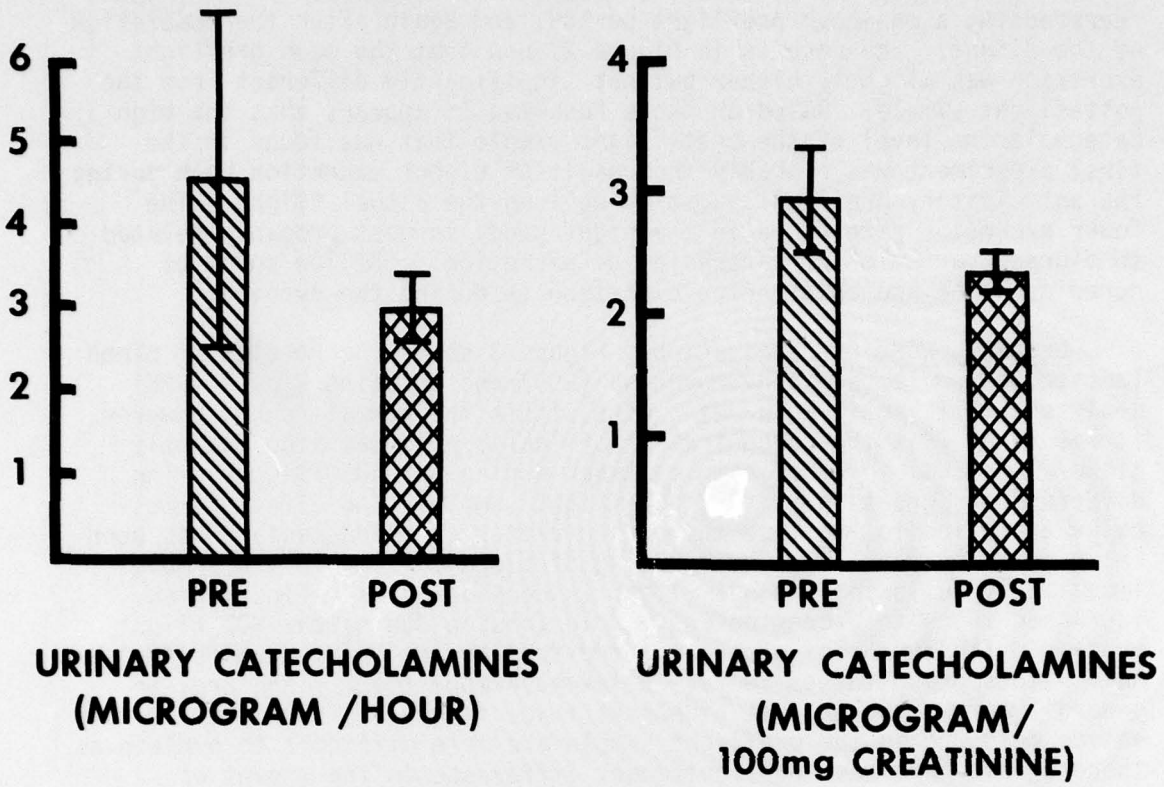
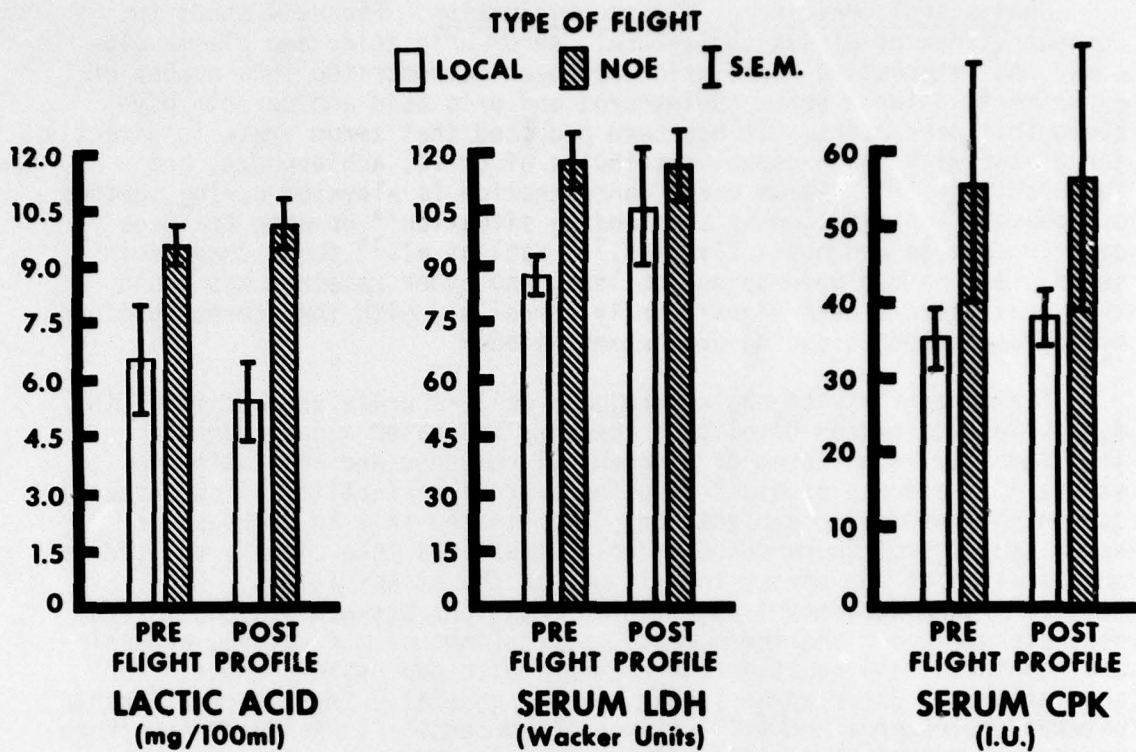


Figure 2. Urinary catecholamine excretion during night nap-of-the-earth flights. SEM: Standard Error of the Mean is indicated at the top of each bar.



**Figure 3.** Effect of NOE flight on blood lactic acid, serum LDH and CPK. Open bars: "normal" or routine flight at 1,000 feet AGL. Shaded bars: nap-of-the-earth flight at or below treetop level. SEM: Standard Error of the Mean is indicated at the top of each bar.

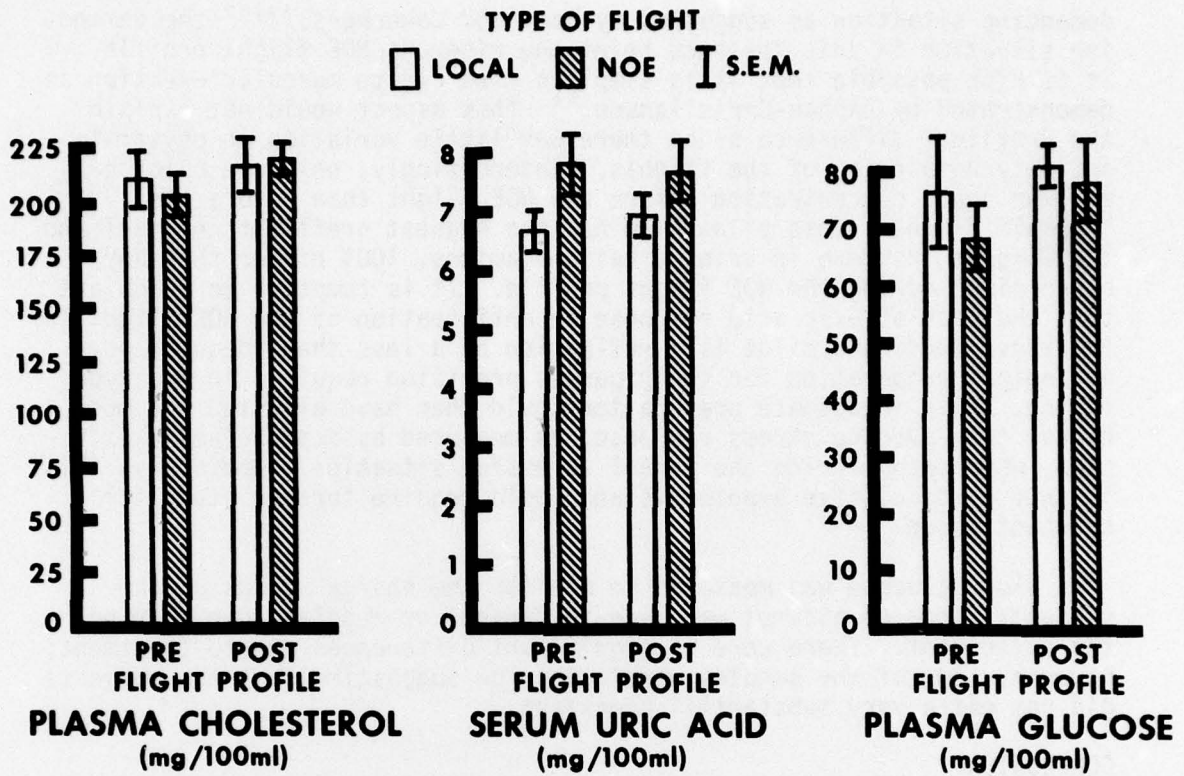
Cholesterol, Uric Acid, Glucose Evaluation. Figure 4 shows the concentrations of plasma cholesterol, serum uric acid, and plasma glucose. An interesting correlation has been demonstrated in a number of experiments between serum cholesterol and uric acid and certain psychological parameters. It has been proposed that serum urate is directly correlated with the personal attributes of drive, achievement, and leadership.<sup>3,25,36</sup> Serum urate concentration is elevated during periods of apparent anticipation of a demanding situation<sup>33</sup> or when trainees eagerly take on arduous activities.<sup>34</sup> Kasl et al.<sup>15</sup> found changes in serum urate in men undergoing job loss, and other research has found that urate levels were significantly correlated with the scores obtained by medical students during oral examination.<sup>2</sup>

The variability of cholesterol, as well as urate and cortisol, in men during underwater demolition training indicated a considerable individuality in patterns of biochemical response and adaptation to stress.<sup>40</sup> Peterson et al.<sup>29</sup> also found great variability of cholesterol concentrations within subjects and demonstrated that an increase in serum cholesterol in response to cold stress was more closely related to anticipation of the stress than it was to the stress itself. Rahe et al.<sup>35</sup> found consistent negative correlations between subjects' serum cholesterol levels and their moods and feelings of motivation, arousal and happiness, and positive correlations with depression, anger, fear and lethargy. Often workers have found a generally inverse relationship between cholesterol and self-ratings of success.<sup>2,15</sup> Rahe and coworkers, based on a number of investigations, have stated that serum uric acid elevations are generally seen during anticipatory or excitatory stress whereas serum cholesterol rises are generally seen during ongoing and dysphoric stress.<sup>37</sup> From their work it appears that if one wishes to know if individuals are "primed" to handle an upcoming challenge, one should compare a current serum uric acid to baseline values for that person. On the other hand, if one wishes to assess feelings of burden or failure of men in a stress situation, one should compare a current serum cholesterol to baseline values for that person.<sup>37</sup>

There is disagreement as to the interpretation of these serum changes;<sup>4</sup> however, the intriguing psychological interrelationship with serum cholesterol and uric acid prompted their measurement in this study in pilots flying NOE. Serum cholesterol levels in our experiment were within the normal range. The type of flying had no appreciable effect on cholesterol; however, in both NOE and "normal" flight, cholesterol tended to be higher in the post-flight sample ( $P < .10$ ).

Serum urate levels were also within the normal range. There were no significant differences between the pre- and post-flight samples; however, the samples taken during NOE flying were significantly higher ( $P < .05$ ) than during the higher altitude local area flight. This could be a biochemical reflection of a response to the anticipation of a





**Figure 4.** Effect of NOE flight on plasma cholesterol, serum urate and plasma glucose. Open bars: "normal" or routine flight at 1,000 feet AGL. Shaded bars: nap-of-the-earth flight at or below treetop level. SEM: Standard Error of the Mean is indicated at the top of each bar.

demanding situation as suggested by Rahe and coworkers;<sup>33,37</sup> the demanding situation in this instance being the rigorous NOE flight profile. It is also possible that it is simply a rise due to muscular exertion as demonstrated by Zachau-Christianson.<sup>46</sup> This aspect would not explain the preflight difference since there was little variation in physical activity before any of the flights. Interestingly, only one pilot had a lower urate concentration before the NOE flight than before the "normal" flight. This pilot also had the highest preflight cortisol and the largest increase in urinary catecholamines, 100% higher than any other pilot during the NOE flight profile. It is tempting to speculate that the lack of uric acid response in anticipation of the NOE flight in the above mentioned pilot is a reflection of a less than adequate psychological preparation for the rigorous precision required in NOE type flying. This inadequate preparation could then have elicited the much higher than average stress response, as measured by catecholamine excretion, when encountering the actual stressful situation. Obviously, this is only a speculative hypothesis and would require further study for substantiation.

Blood glucose was measured to monitor the energy status of the subjects since no attempt was made to control or regulate diet during the experiment. There were no significant differences due to treatment; however, some of the samples were quite low suggesting that the subjects did not eat a very substantial breakfast.

#### CONCLUSION

The NOE flight resulted in significantly higher urinary catecholamine excretion ( $P < .05$ ), serum uric acid ( $P < .05$ ) and blood lactic acid ( $P < .01$ ). Preflight cortisol was significantly higher than post-flight ( $P < .01$ ), and post-flight catecholamine excretion rate was higher than during the three hour post-flight sample period ( $P < .01$ ). The biochemical results are consistent with the reports that NOE flight is physically more demanding in terms of muscular strain. The increased catecholamine excretion may indicate the perception of NOE flight as a more demanding and stressful activity than flight at higher altitudes. In light of previous work, the higher serum uric acid levels prior to NOE flight may indicate the pilot's psychological preparation and possible performance during NOE flight.

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 THE EFFECT OF NAP-OF-THE-EARTH (NOE) HELICOPTER FLYING ON  
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