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THE EFFECT OF +GX ACCELERATION ON PLATELET ACTIVATION
AS DETERMINED BY PL. (U) AIR FORCE AEROSPACE MEDICAL
RESEARCH LAB WRIGHT-PATTERSON AFB. F S CRAMER ET AL.
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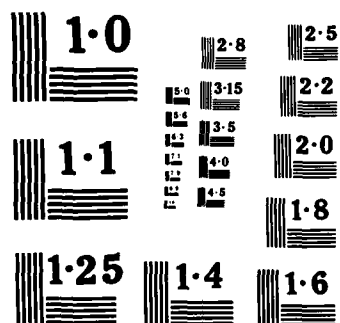
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**THE EFFECT OF +G_x ACCELERATION ON PLATELET
ACTIVATION AS DETERMINED BY PLASMA
PLATELET FACTOR 4 LEVELS**

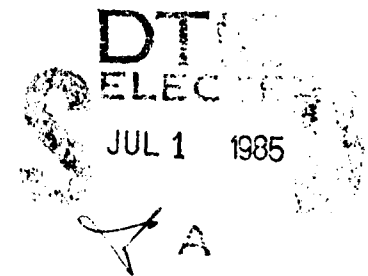
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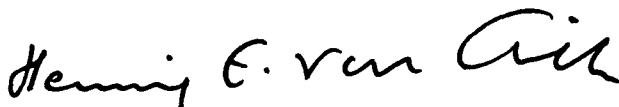
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The voluntary informed consent of the subjects used in this research was obtained as required by Air Force Regulation 169-3.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

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FOR THE COMMANDER



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Six healthy men were exposed to a +Gx acceleration (chest to back) profile with peak +Gx levels of 5, 8, and 9 G. Platelet factor 4 levels and platelet counts were measured prior to and immediately following the acceleration exposure. The +Gx acceleration exposure did not affect either parameter, however elevated pre-acceleration platelet factor 4 levels were found in five of six subjects. The possible significance of this is discussed,					
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FIGURE LEGEND

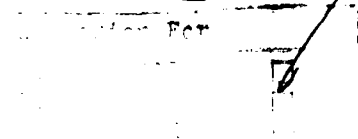
Figure 1

+Gx Acceleration Profile

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A-1

INTRODUCTION

Future space missions will expose man to unique combinations of environmental stresses. Decompression and +Gx acceleration exposure is one such combination. It is important to determine if there are some features of these stresses which by themselves are benign, but in combination can result in problems for the astronaut.

Evidence exists implicating platelets and platelet activation as a factor in the pathogenesis of decompression sickness. Light and electron microscopic studies show platelets adhering and aggregating to a protein matrix that surrounds the intravascular bubbles (7,10). Rats with thrombocytosis have a higher incidence of decompression sickness than control rats (2). Platelet counts are shown to decrease with decompression sickness (2,5), suggesting that platelets are being consumed during decompression. Finally, when rats are exposed to explosive decompression and given infusions of adenosine diphosphate (ADP), a stimulant of platelet aggregation, the interval between the decompression and the occurrence of hypotension and death is shortened (11).

Recently several groups have reported that platelets are activated in normal, healthy subjects following exercise (9,14,15). One method used to quantify platelet activation is to determine platelet factor 4 serum concentrations (13).

+Gx acceleration (chest to back) provides a significant physical stress in man. At acceleration levels greater than +4 Gx, the subject experiences chest pain. Breathing becomes difficult, causing the subject to become dyspneic and to breathe using his abdominal muscles. Above +6Gx, pulmonary volumes are reduced, and the subject is unable to move his arms and legs (1,3).

The purpose of this study was to determine whether +Gx acceleration, using an acceleration profile proposed for future space launches, causes platelet activation as determined by platelet factor 4 concentrations.

METHODS

Centrifuge

The experiment was conducted on the Dynamic Environment Simulator (DES), a 3 axis, 19 foot radius, man-rated centrifuge located at the Air Force Aerospace Medical Research Laboratory, Wright-Patterson AFB, Ohio. The acceleration profile consisted of 3 +Gx peaks of 5, 8, and 9 G over five minutes (figure 1).

Subjects

Six healthy, active duty Air Force men, ranging from twenty-four to thirty-six years of age, were used in the experiment. Four subjects were members of the centrifuge human subject panel and had passed a rigorous qualifying medical examination consisting of a history, physical, and laboratory examinations including EKG and stress EKG. Two months following the experiment, one subject was found to have adult onset diabetes, requiring 20 units NPH insulin for control. He had no sign of micro- or macrovascular disease at the time of diagnosis. Two subjects were aircrew members and did not have a stress EKG as part of their initial examination. Four subjects had less than five rides on the centrifuge, and four subjects had had no previous exposures to +Gx acceleration.

All subjects were briefed concerning the hazards of the experiment and signed consent forms. Specifically the subjects were told that: they would have difficulty breathing, they might develop petechiae, become motion sick, or develop a cardiac arrhythmia during the acceleration exposure. The subjects did not take any medication known to interfere

with platelet function for at least two weeks prior to the experiment. One subject was taking minocycline for acne.

Data Collection

Blood pressure was measured and venous blood samples were collected prior to and following (within 2-3 minutes) the acceleration exposure. An EKG rhythm strip was recorded prior to, during, and following the acceleration exposure. When it was found that the pre-acceleration platelet factor 4 blood levels were elevated, blood was collected on the subjects at rest. Due to scheduling difficulties, only four subjects had a resting blood sample drawn.

Platelet Factor 4 assay

Blood was collected from the subjects through a 20 gauge needle by a clean venipuncture in a single attempt. Blood was first collected in an EDTA tube and used for a platelet count determination using a Coulter model 5 plus electronic cell counter. This counter measures platelets accurate to $\pm 1 \times 10^3$ cells/mm³. The second blood sample was collected in a Thrombotect^R tube (Abbott Laboratories, North Chicago, IL.). The tube was immediately inverted, then rapidly placed in an ice bath. After 30 minutes in the ice bath, the tube was centrifuged at 2500 G for 20 minutes, after which the plasma was transferred to another tube and frozen at -20° C for up to six weeks prior to analysis.

Platelet factor 4 (PF₄) levels were determined by radioimmunoassay using a commercial kit (Abbott Laboratories, North Chicago, IL.) with a reported normal range of 0 to 10.4 ng/ml. PF₄ levels were determined in duplicate.

Statistical Analysis

In analyzing the change from pre- to post acceleration, problems with lack of normality occurred. Instead of performing t-tests on suitable

transformations of the data, the Wilcoxon signed rank test was used. In all cases, the null hypothesis $H_0: \Delta=0$ was tested against the alternative hypothesis $H_a: \Delta \neq 0$. With only 6 subjects, it is difficult to make substantial conclusions. However, with the Wilcoxon test, all 6 subjects would have to change in the same direction to achieve significance ($p_{.05}$).

RESULTS

Table 1 shows the maximum heart rates and blood pressures for the subjects. No significant changes were found between the pre- and post acceleration blood pressures and heart rates (Table 1). The mean subject heart rate during the acceleration exposure increased by 28 beats per minute. This increase was significant ($p=0.031$).

Platelet factor 4 levels and platelet counts did not change following the +Gx acceleration (Table 2). Five of six subjects had elevated pre-acceleration PF_4 concentrations if compared to reported normal levels of 0-12. Three subjects, who had elevated pre-acceleration PF_4 levels, had either normal baseline controls or a baseline control dramatically lower than the pre-acceleration level. Subject 4 was the subject who developed adult onset diabetes. His PF_4 levels were similar to the mean values, and no change in his platelet factor 4 level was found following the acceleration exposure.

DISCUSSION

The subjects' platelet counts did not change following the acceleration exposure. Previous studies have found that following strenuous exercise, the platelet count becomes elevated (4,12). This fact and the finding that the mean peak heart rate during the +Gx acceleration was only 99, suggests that the +Gx acceleration profile was not as physically stressful as anticipated. Another study performed in our laboratory, using

the same acceleration profile, found the maximum heart rate to be 102 and the maximum respiratory rate 35. +Gx acceleration is significantly stressful to the pulmonary system, but only mildly stressful to the cardiovascular system.

Platelet factor 4 plasma concentrations were not increased following the acceleration exposure which suggests that the proposed +Gx acceleration does not activate platelets. Another possible explanation is that pre-acceleration platelet factor 4 elevation masked any increase in platelet factor 4 activity resulting from the +Gx acceleration. Since the maximum concentration the assay was capable of measuring was 100 ng/ml, well above the platelet factor 4 levels measured in this study, this second possibility seems less likely.

+Gx acceleration may enhance the effect of decompression by other mechanisms. For example, +Gx acceleration compresses the astronaut's tissues. This compression may increase the release of gas bubbles from the tissues, thereby providing some micronuclei which can grow rapidly under hypobaric conditions.

Five of six subjects had elevated platelet factor 4 levels prior to the acceleration. This finding was unexpected. Resting baseline values were found to be normal or nearly normal for four subjects who had elevated pre-acceleration platelet factor 4 levels. In addition, there is no reason to believe that the other two healthy subjects would have elevated resting baselines. Several explanations are possible. Laboratory error is one possibility. Difficulties with the platelet factor 4 assay have been reported (8). However, similar determinations were made using identical samples on two occasions by different technicians.

Another possible explanation is that subject anxiety may have caused platelet activation. Five of the six participants were inexperienced centrifuge subjects. This +Gx acceleration exposure was the first +Gx acceleration exposure for all the subjects with elevated pre-acceleration platelet factor 4 levels. In addition, all subjects were carefully briefed about what to expect, i.e., chest pressure, difficulty breathing, etc. All these factors would undoubtedly contribute to pre-run anxiety and possibly elevate catecholamine levels. Epinephrine is a well known, potent platelet activator (17). The only subject who had normal pre-acceleration platelet factor 4 levels had had two prior exposures to this +Gx acceleration profile. Unfortunately, attempts to measure plasma epinephrine and norepinephrine levels from the remaining serum were unsuccessful. The finding that anxiety may cause platelet activation has profound implications in the genesis and treatment of decompression and myocardial infarction.

Subject 4, the adult onset diabetic, had platelet factor 4 levels similar to the other subjects. Others have found that the majority of diabetics have normal levels of platelet factor 4 (16) or platelet factor 4-like activity (6).

In summary, the platelet count or platelet factor 4 levels did not change in subjects following +Gx acceleration. Elevated pre-acceleration platelet factor 4 levels were found and might be due to subject anxiety.

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TABLE 1. Subject's Blood Pressures and Heart Rates

(Prior to, During and Following + G(x) Acceleration)

Subj No.	BP Systolic(mmHg)		BP Diastolic(mmHg)		Heart Rate(beats per min.)		
	pre-Gx	post Gx	pre-Gx	post Gx	pre-Gx	during	post Gx
1	128	134	80	84	66	90	59
2	108	118	70	72	65	91	71
3	142	150	88	92	106	119	105
4	128	122	66	74	50	83	62
5	122	126	74	70	95	120	85
6	120	136	82	80	44	90	39

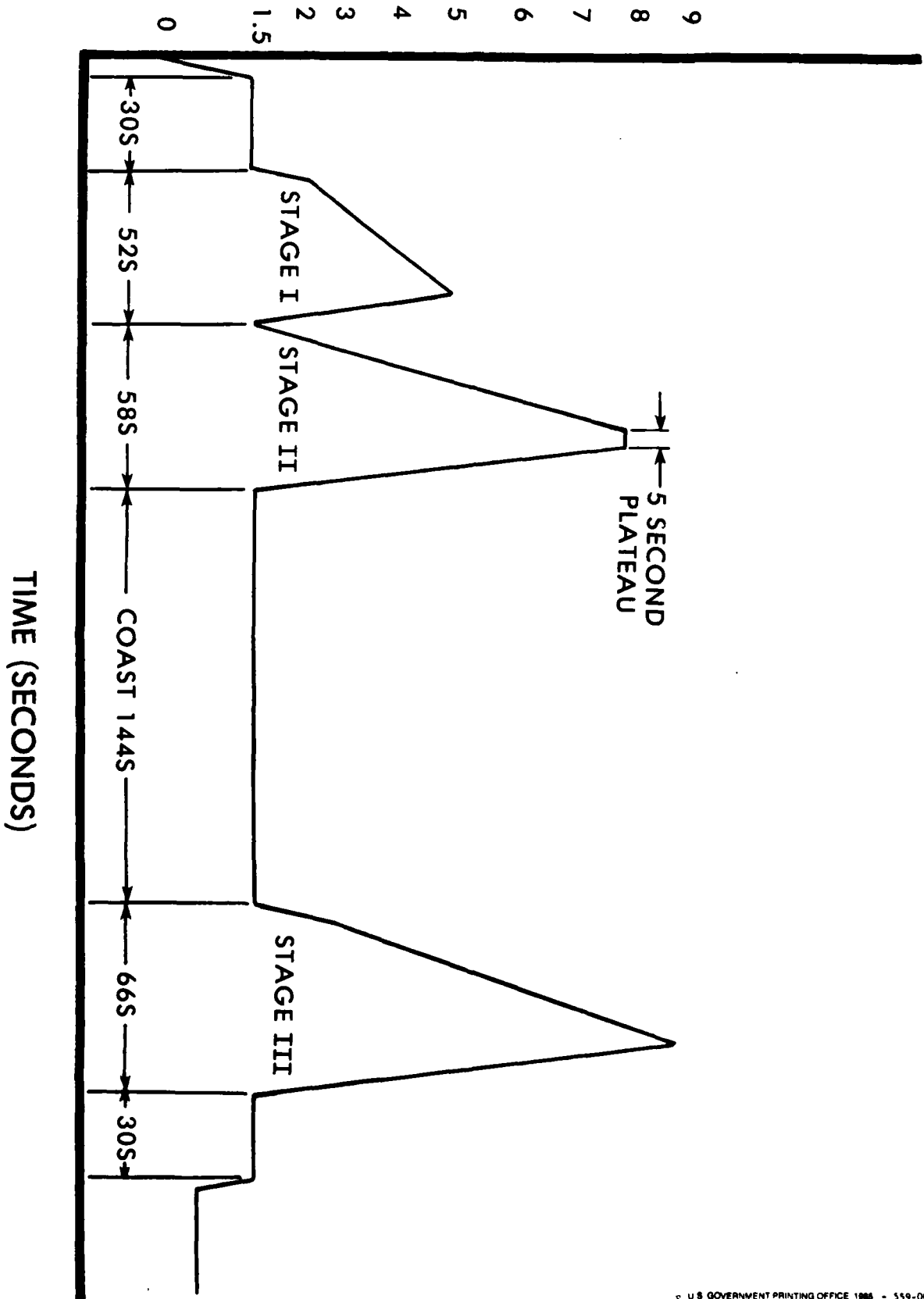
means ±S.E.	125±11	131±12	77±8	79±8	71±25	99±16*	70±23

*p=.032 when compared to pre- or post

Table 2. Subject's Platelet Factor 4 Concentration and Platelet Counts
(Prior to and Following +G(x) Acceleration)

Subj No.	Platelet Factor 4 (ng/ml)			Platelet Counts ($\times 10^3/\text{mm}^3$)		
	Resting Baseline	pre-Gx	post Gx	Resting Baseline	pre-Gx	post Gx
1	-	17	16	-	227	209
2	10	7	9	145	158	149
3	-	33	36	-	293	276
4	18	58	63	197	201	185
5	7	34	33	168	176	182
6	5	63	45	186	225	223
means \pm S.E.		35 \pm 22	34 \pm 20		213 \pm 47	204 \pm 43

Gx ACCELERATION (G)



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