AD-A075 006 CALIFORNIA UNIV DAVIS F/G 6/19
CUMULATIVE ASPECTS OF REPEATED HSG EXPOSURE.(U)
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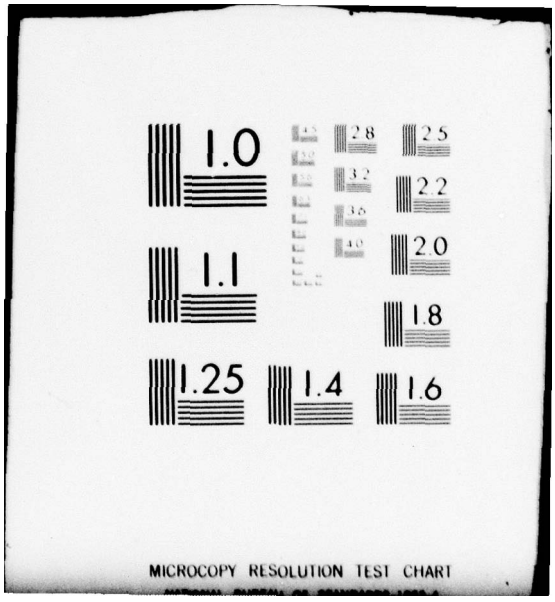
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Report SAM-TR-79-17

LEVEL II

CUMULATIVE ASPECTS OF REPEATED HSG EXPOSURE

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July 1979

Final Report for Period 1975 - 1977

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Prepared for
USAF SCHOOL OF AEROSPACE MEDICINE
Aerospace Medical Division (AFSC)
Brooks Air Force Base, Texas 78235

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NOTICES

This final report was submitted by The University of California at Davis under contract F41609-76-C-0012, job order 7930-12-14, with the USAF School of Aerospace Medicine, Aerospace Medical Division, AFSC, Brooks Air Force Base, Texas. Dr. Russell R. Burton (USAFSAM/VNB) was the Laboratory Project Scientist-in-Charge.

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The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act of 1970 and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources - National Research Council.

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

This technical report has been reviewed and is approved for publication.

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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: SAM-TR-79-17
2. GOVT ACCESSION NO.
3. RECIPIENT'S CATALOG NUMBER

4. TITLE (and Subtitle): Cumulative Aspects of Repeated HSG Exposure,
5. TYPE OF REPORT & PERIOD COVERED: Final Report, 1975-1977
6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s):
A. H. Smith, Ph.D., Dept of Animal Physiology
E. A. Rhode, Ph.D., Dept of Medicine--Vet
W. L. Spangler, Ph.D., Dept of Vet Pathology

8. CONTRACT OR GRANT NUMBER(s): F41609-76-C-0012 new

9. PERFORMING ORGANIZATION NAME AND ADDRESS:
University of California, Davis
Davis, California 95616

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS:
62202F
7930-12-14

11. CONTROLLING OFFICE NAME AND ADDRESS:
USAF School of Aerospace Medicine (VNB)
Aerospace Medical Division (AFSC)
Brooks Air Force Base, Texas 78235

12. REPORT DATE: July 1979

13. NUMBER OF PAGES: 77

14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)

15. SECURITY CLASS. (of this report): Unclassified

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)
Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

pathology	acceleration
domestic fowl	aerial combat maneuvers
pathophysiology	avian physiology

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

A system is described for acceleration treatment of domestic fowl. A reasonable end point for acceleration tolerance is provided by a bradycardia that occurs fairly close to the lethal limit. In a group of 61 male Rhode Island Red chickens exposed to +6 G_z, the mean tolerance (± SD) was 11.1 ±10.6 minutes. Among individuals, the acceleration tolerance was inversely related to both body size and age, and positively to the pretreatment heart rate.

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Block 20. Abstract (Cont'd)

In some individuals the acceleration induced cardiac lesions (subendocardial hemorrhage or congestion); and at the subcellular level, further degenerative changes became apparent, including regions of hypercontraction of cardiac myocytes, mitochondrial swelling, and necrosis in some cells. Discrete regions of fibrous tissue proliferation and regions with increased frequency of intercalated discs were also observed. The latter was considered an adaptive reinforcement of the pathologically altered myocardium. With repeated HSG exposure (4 minutes at +6 G_z, 8 times daily for 6 months), evidence of any subendocardial hemorrhage was no longer apparent, but the submicroscopic changes remained.

In other experiments, chickens were given a single 1-minute exposure to a field that increased from 5 to 18 G_z for the series. Acceleration-induced changes were observed in heart rate during and after the treatment, and subsequently in lymphocyte frequency and body-mass maintenance. Generally, bradycardia and lymphopenia increased proportionally to field strength. Above 13 G, normal growth and even maintenance of pretreatment body mass were impaired.

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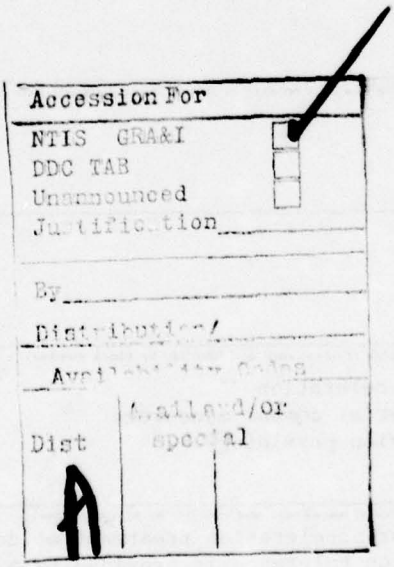

CONTENTS

FIGURES (continued) Page

TABLES

CUMULATIVE ASPECTS OF REPEATED HSG EXPOSURE

BACKGROUND

The physiological effects of radial acceleration upon pilots of maneuvering aircraft have been an aviation problem since the 1930s, and these have been abundantly investigated. Several comprehensive reviews of the early research are available (13-15,17,30). With the increasing capabilities of modern aircraft, interest has shifted towards the upper tolerance limits, and this region (>6 G[1] for 15 seconds) has been designated high sustained G (HSG) (6,8). It has become particularly important to develop animal models for human HSG responses in order to understand the pathophysiological consequence of overexposure. The objective of this research was, in laboratory animals, to identify and describe changes at the cell/tissue level resulting from repeated exposure to HSG, and to estimate their probable hazard and reversibility.

The domestic fowl provides a reasonable human model for acceleration studies. It is a biped, and although the body is not strictly vertical, as man's, its vasculature has similar basic characteristics. Since the principal blood vessels are generally parallel to the field of gravity (normally $+G_z$, as compared to $-G_x$ for quadrupeds), its circulation is significantly affected by gravity-induced intravascular hydrostatic pressures. Consequently it has developed a visceral vasomotor apparatus--as has man. Quadrupeds, with a principal vasculature perpendicular to the field of gravity, are less susceptible to intravascular hydrostatic pressures and lack a visceral vasomotor apparatus. So, from the standpoint of circulation and hemodynamic acceleration susceptibility, the chicken resembles humans more closely than do other common experimental animals such as rats and dogs.

Commercial breeds of chickens are readily available in genetically standardized lines, with convenient body size (1.8 to 3 kg), and at a low unit cost. They are large enough to be reasonably susceptible to acceleration, yet small enough to permit simultaneous acceleration of several subjects. Birds (which, like mammals, are homoiotherms) appear to share some general pathological changes with a number of animal species when subjected to hypovolemic (hemorrhagic) shock. These include congestion and necrosis in the liver, hemorrhage in the lung, intestinal congestion and hemorrhage, acute renal tubular necrosis, subendocardial hemorrhage, and early degenerative changes in the myocardium (11,16). The mechanisms responsible for these changes are not fully understood at this time, but similar pathological tissue alterations have been produced by HSG (4,7). So the tissue responses of the fowl support its usefulness as a human model for acceleration studies. Also, chickens have been employed as subjects in chronic restraint (1,2,5) and chronic acceleration (28, 29) studies, which provide a valuable perspective in the interpretation of HSG results.

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[1] G, throughout this manuscript, will be defined as $+G_z$ (positive acceleration force).

The principal objective of this research was to evaluate the myocardial pathology resulting from repeated exposures to HSG, administered several times daily over a long period of time (6 months to 1 year). Some preparatory investigations were necessary to determine tolerable G-levels and exposure times suitable to the intended treatment schedule. Observations made during preparatory investigations led to a variety of secondary experiments--such as screening the groups of experimental animals to eliminate susceptible individuals, using conditioning exposures to enhance tolerance, and determining maximum field tolerance for rather short exposures. Consequently, the results reported herein tend to provide a rather broad description of the acceleration biology of the fowl, and may have a usefulness beyond the specified objective.

METHODOLOGY

Implementation of this research required adapting an available centrifuge to accelerate chickens and developing an animal restraint harness suitable to HSG treatments. The centrifuge was designed by S. J. Sluka and built for Professor Nello Pace (University of California, Berkeley) to reproduce launch and recovery acceleration profiles for biosatellites. It was readily adapted to our purpose, with minor changes in the hydraulic-drive system and in the animal carrier.

Animal Centrifuge

The centrifuge used for these experiments is a hydraulic-driven apparatus with a 20-G capacity. Plan and side views of this machine are shown in Figures 1 and 2. Power to the centrifuge is provided by a hydraulic system, shown schematically in Figure 3. The rotation of the centrifuge is regulated by a valve on the hydraulic pump output, which is operated by a throttle. An electric tachometer is operated by a cogwheel that engages the centrifuge drive chain (Fig. 2), and its output is connected to a galvanometer gauge that is mounted near the hydraulic controls. This gauge is calibrated in G's; however, because of the exponential nature of the operational characteristics (Fig. 4), it is not accurate at higher fields. For precision adjustment, the rotation rate is adjusted by counting the time required for 10 revolutions, and the appropriate throttle-opening position is fixed with a set screw.

The onset rates of the centrifuge are not great--generally in the order of 1 G per second--as shown in the field-time curve in Figure 5a. The relationship is exponential, with the kinetics:

$$G_t = e^{kt}$$

where: G_t is the field strength, being G after t seconds.

The first derivative of the curve, the onset-rate curve, is shown in Figure 5b.

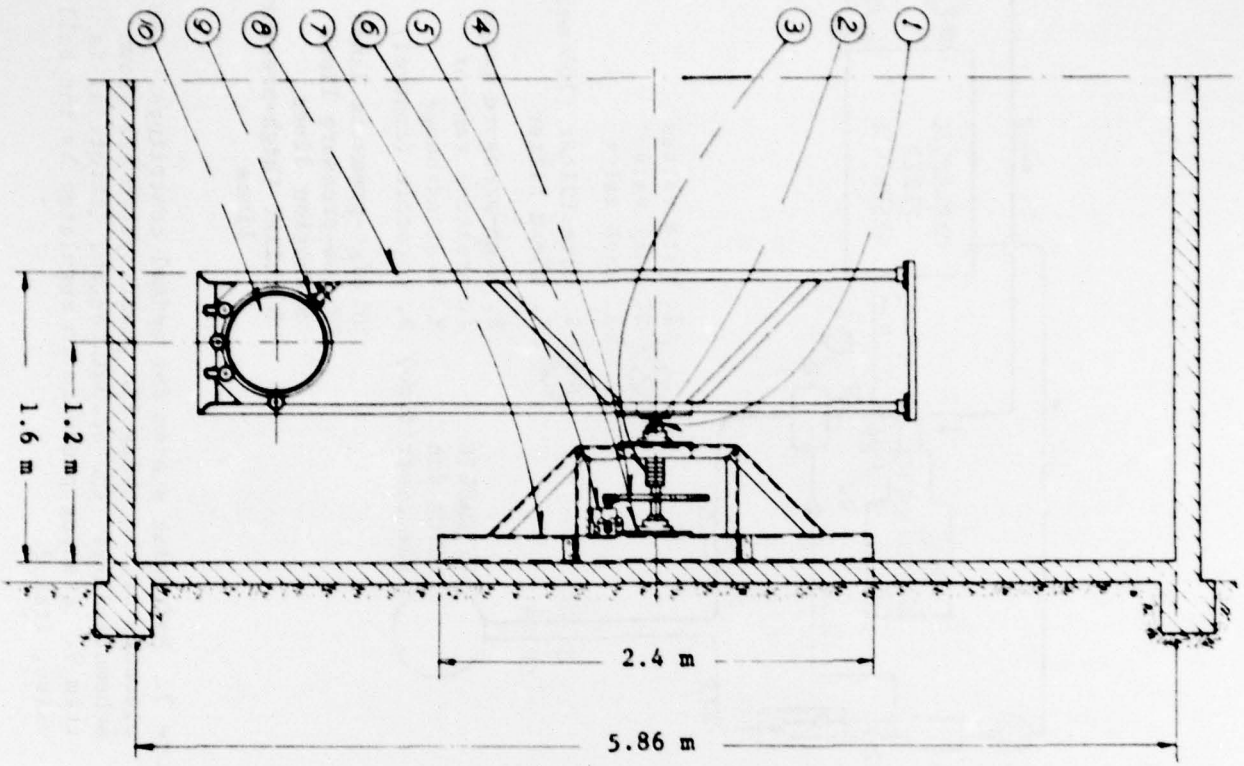


Figure 1. Central cross-section of the animal centrifuge. Items identified are: (1) centrifuge spindle; (2) upper bearing; (3) sliprings for power transfer; (4) large sprocket--centrifuge drive; (5) lower bearing--thrust bearing that supports the turntable; (6) hydraulic motor; (7) base frame; (8) rotating centrifuge arm; (9) mounting rollers; and (10) animal carrier. The silver-silicon slipring system for signal transfer, which extends to the ceiling from the central spindle, is not shown, nor the oil pump and oil reservoir for hydraulic power which are located in an adjacent room.

The counterbalance arm has a heavy steel plate that compensates for the load developed in the animal-carriage arm during operation. For added loads, the counterbalance plate has a rod upon which 1.27-cm steel discs can be mounted, and locked in place by a restraining collar secured by an allen screw. The six plates available have the following characteristics:

Plate No.	Diameter (cm)	Actual mass (kg)	Counterbalancing mass (kg)	(lb)
1	10.4	0.72	0.45	1
2	14.1	1.43	0.91	2
3	19.4	2.87	1.81	4
4	25.7	5.01	3.17	7
5	30.5	7.17	4.54	10
6	43.1	14.33	9.07	20

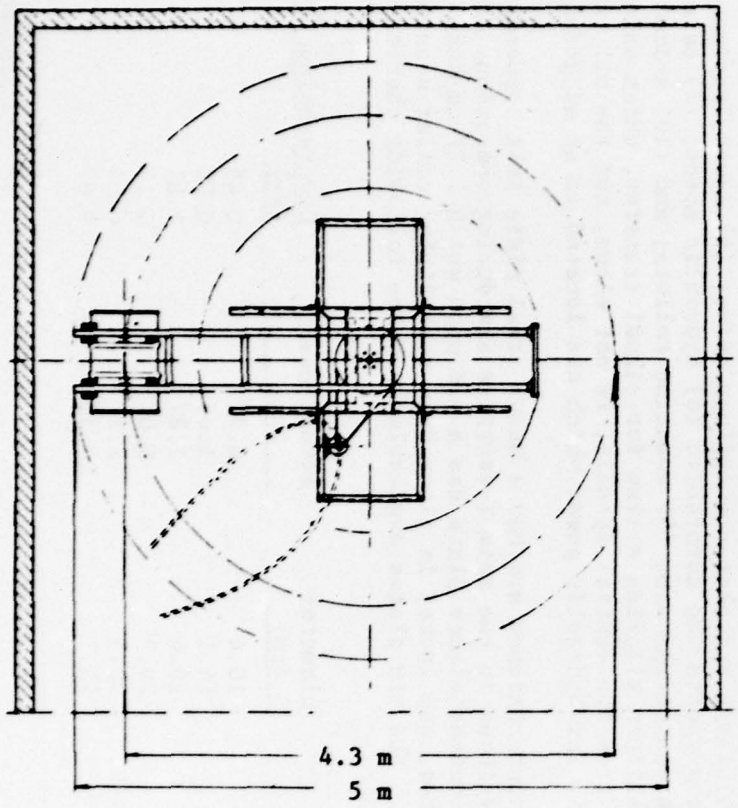


Figure 2. Animal centrifuge plan view. The dashed lines indicate hydraulic fluid lines, connecting the hydraulic motor (on frame) to the hydraulic pump which is in an adjacent room.

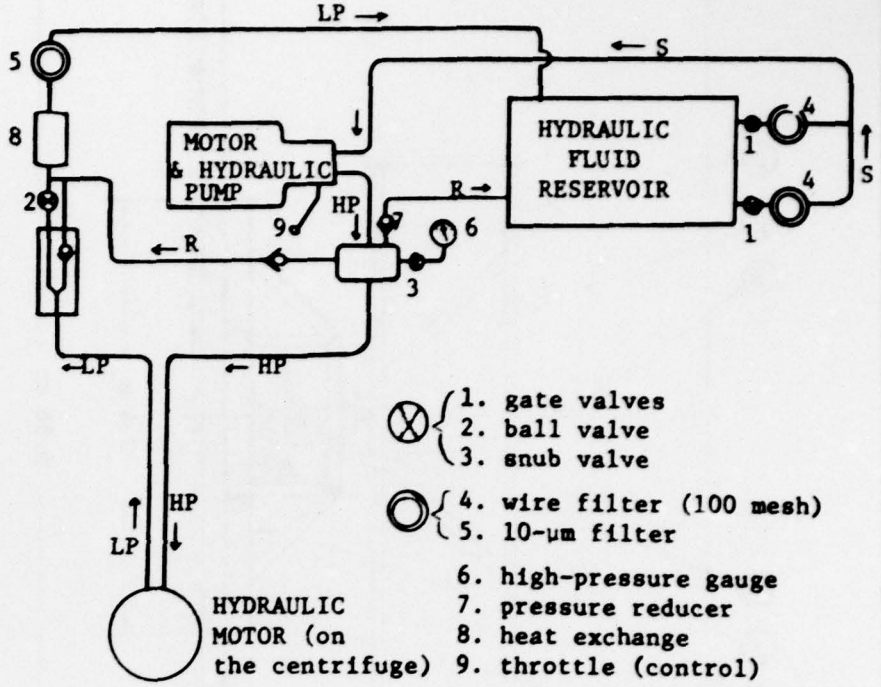


Figure 3. Hydraulic system for animal centrifuge. The elements of the hydraulic power system are shown schematically. The output control (throttle) is item 9. Braking pressure is regulated by the ball valve, item 2.

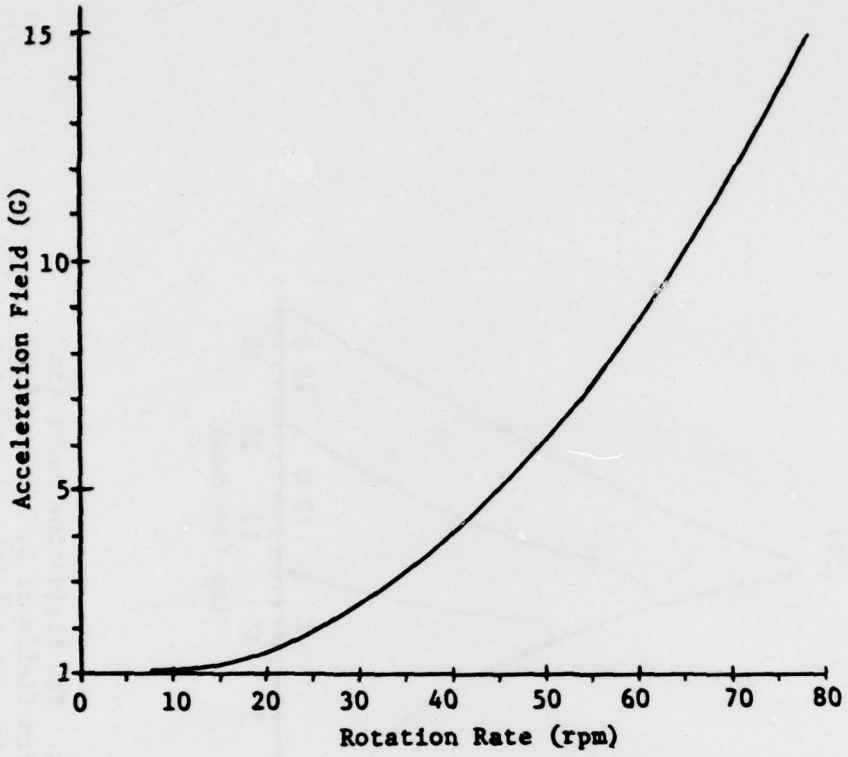


Figure 4. Performance characteristics of the animal centrifuge. Acceleration fields (resultants of centrifugal forces and gravity) at center of animal carrier (2.1-m radius) are indicated for the following operating rates:

Net field (G)	Rotation rate (rpm)	Time for 10 revs (sec)
2	27.0	22.2
3	34.3	17.8
4	40.0	15.0
5	45.3	13.2
6	50.0	12.0
7	54.0	11.1
8	57.8	10.4
9	61.3	9.8
10	64.5	9.3
11	67.6	8.9
12	70.5	8.5
13	73.5	8.2
14	76.3	7.9
15	78.8	7.6
16	81.2	7.4
17	83.8	7.2
18	86.2	7.0
19	88.8	6.8

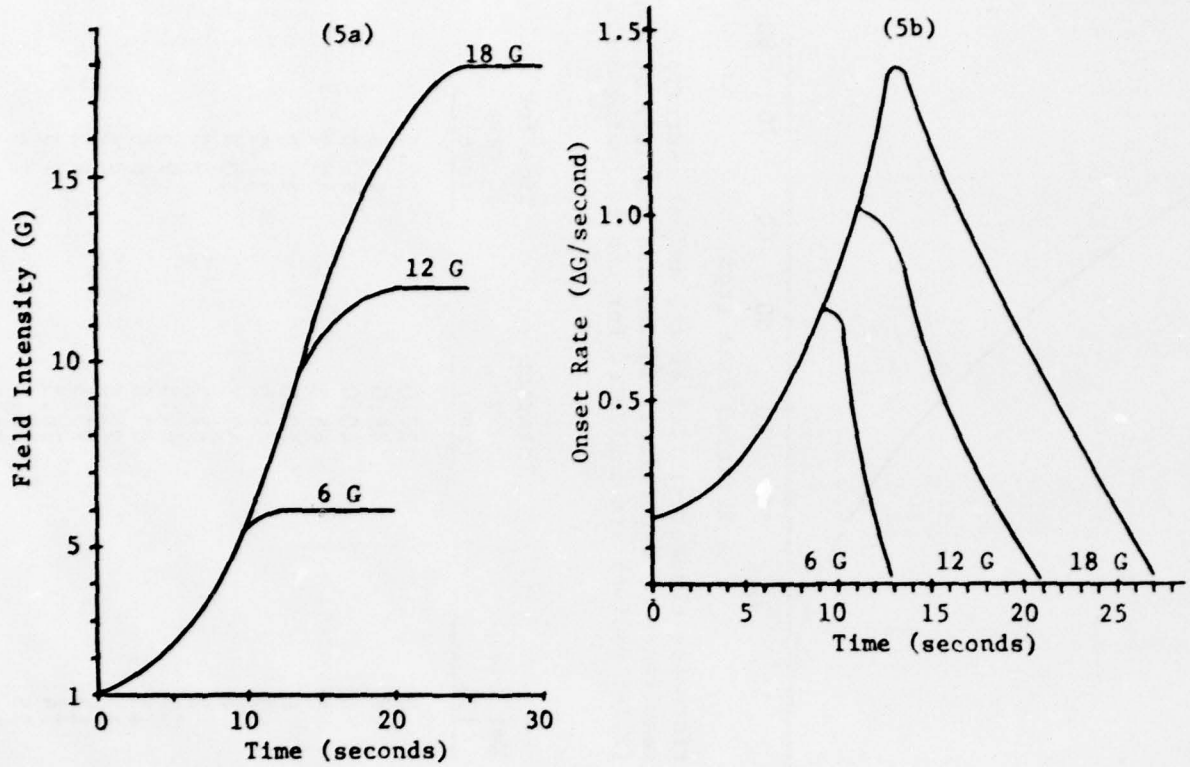


Figure 5. Centrifuge starting characteristics. The field-time curve (5a) describes the time required to attain fields of 6, 12, or 18 G. The first derivative of the field-time curve is the onset-rate curve (5b), indicating rates of change in field strength.

The slowing curve (Fig. 6) is biphasic, both components of which are exponential:

$$G_t = G_o e^{-kt}$$

where: G_o is the initial field intensity--or the zero-time intercept of the component.

The early component has the rate constant -0.39 (a half-time of 1.8 seconds), and this is the same for all slowing curves shown in Figure 6. It persists until the centrifuge slows to a field of 5 or 6 G, and is not perceptibly changed whether the hydraulic brake (ball valve, Fig. 3) is on or off. This appears to be determined by air resistance, which is arithmetically related to field strength (since both G and air pressure are related to V^2):

$$\text{Air pressure (kg)} = 0.036\ (G - 1)$$

where: Air pressure is the total force on the animal carrier (which has a 0.26 m^2 area).

After the centrifuge has slowed to 5 or 6 G, then resistance in the hydraulic system is the principal factor in slowing the centrifuge. In this phase of slowing, which also is exponential, the rate constants (with t in seconds) are:

	k	$t_{1/2}$
with brake:	-0.058	11.8 seconds
without brake:	-0.045	15.3 seconds

These functional characteristics appear to be much slower than those cited for human centrifuges. However, with the relatively longer exposure times, and the nature of the observations, these characteristics do not appear to be critical.

Animal Carriage

The animal carriage was prepared from a 55-gallon oil drum, which can be removed quite readily from the centrifuge by loosening the mounting rollers. This permits the preparation and rapid interchange of several carriages, each suited to a different purpose. For most trials, a carriage mounted with a rack holding 6 harnessed birds is used. During centrifugation, the principal support is provided by a "vertical" attachment (i.e., parallel to the net acceleration field). A perch also is provided which allows the animals to control any tendency for body displacement during starting and stopping.

The centrifuge turntable is connected to a system of low-noise sliprings (manufactured by Superior Carbon Products), which permits transmission of up to 24 channels of information from the operating centrifuge. Currently an 8-channel, Type R Dynograph is being used to record EKGs on up to 3 birds simultaneously.

Animal Restraint

The experimental birds are exposed to an acceleration field along the $+G_z$ axis, and this requires special support for the neck. Some preliminary HSG

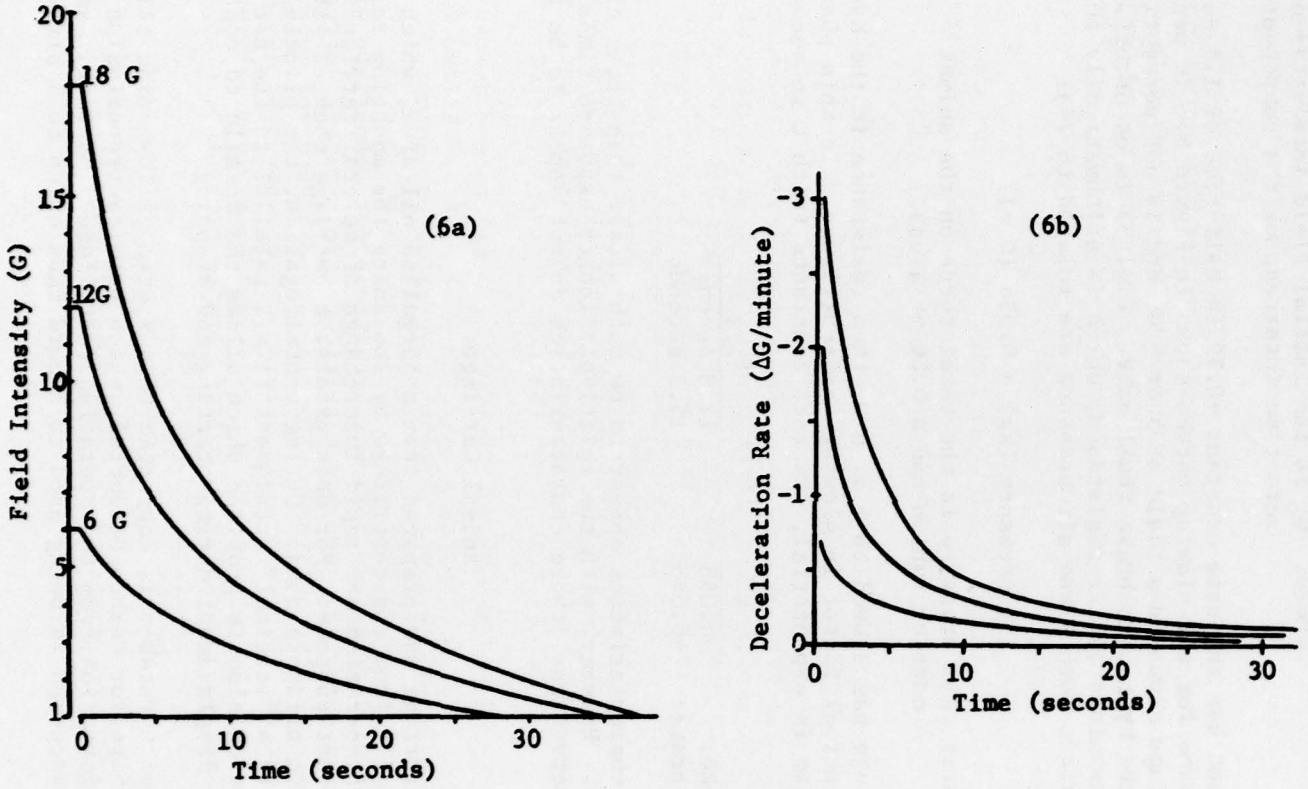


Figure 6. Centrifuge slowing characteristics. Field-time curves for the decelerating centrifuge are shown in 6a. These curves are complex, and the components are described in the text. The deceleration rate (first derivative of the field-time curve) is shown in 6b.

trials indicated that a new support system was necessary, and a restraint harness was designed that encloses most of the bird--with only the legs protruding (Fig. 7). It is made with two outer layers of nylon and an inner layer of 1.3-cm foam rubber. This arrangement not only distributes forces during centrifugation, but also prevents movement of the bird within the harness. Fasteners made of Velcro provide a snug fit and also allow rapid securing and release.

Three rings along the back are used to attach the harness to the centrifuge carriage, maintaining a $+G_z$ orientation of the body to the accleration field (Fig. 8). Currently, birds are attached to the centrifuge in a normal posture, with the principal body axis 30° from horizontal (the plane perpendicular to the acceleration field). At some future time, the influence of the body orientation to the acceleration field upon HSG tolerance may indicate a different orientation, which can be readily arranged.

The harness has been prepared in three sizes: large, for the Rhode Island Red cocks, 3-5-kg body mass; medium, for Rhode Island Red hens and White Leghorn cocks, 2-3kg; and small, for the White Leghorn hens, 1-2-kg.

Experimental Animals

Male Rhode Island Red chickens were selected as subjects because they were readily available in genetically uniform stocks from local commercial sources. They were procured in groups of 200-day-old cockerels and reared at the laboratory. *Groups of hatchmates* were designated serially in order of acquisition as RIR-1, RIR-2, ... etc. At 1 week of age they were dubbed, which prevented the growth of a large comb that might affect the acceleration treatment. They also were debeaked (a commercial practice that limits cannibalism) and inoculated with a vaccine for Marek's disease. These birds are available for experimentation after 90 days of age, at which time they are skeletally mature.

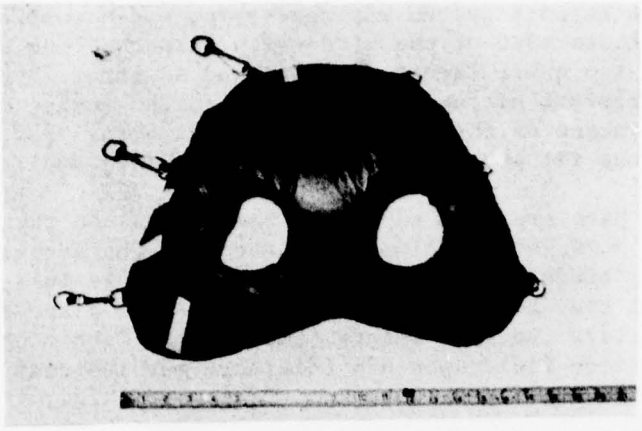
Two strains of White Leghorn chickens are also maintained at our laboratory, one designated WM and the other CA. These also were utilized in these studies to compare acceleration scale effects among mature animals.

Two types of biological controls were available for comparison with the various pathologies observed. One was a static control--merely untreated hatchmates of the experimental birds. The other type of control consisted of animals preconditioned with a minimal acceleration schedule that did not produce physiological change nor any evident gross pathology (see Tables 10 and 11). Other research (2,5) has indicated that simple restraint, of the sort used herein, has no adverse effect on the fowl.

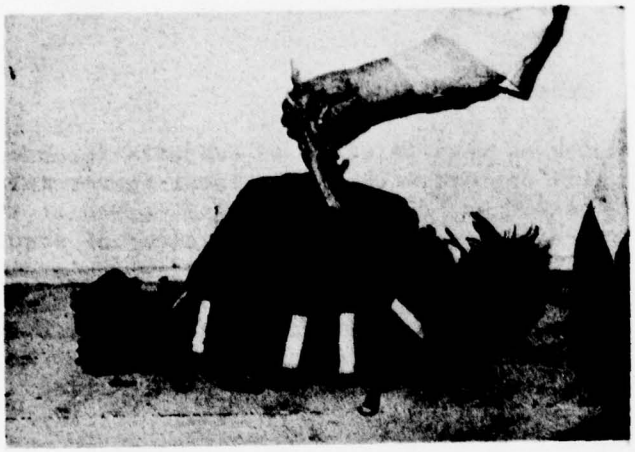
Observations on Centrifuged Animals

Appearance Score

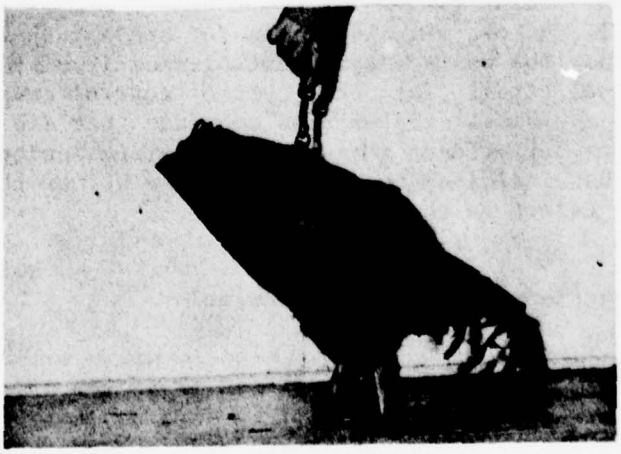
Early in the program, it became apparent that the animals exhibited variable behavior after centrifugation. Since an animal's appearance could be related to its acceleration tolerance, a scoring system was devised (by Mr. Kinder, centrifuge operator) to record it:



a. Bottom view, large-size harness.

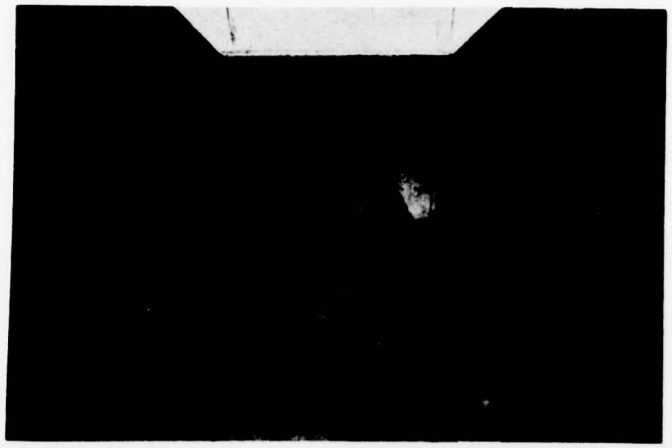


b. Method of putting the harness on the bird. The white stripes are Velcro fasteners,

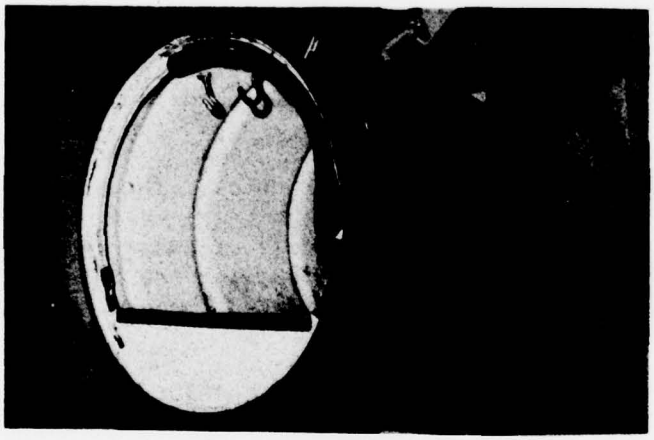


c. Bird installed in harness. Mounting rings connect to load-bearing straps.

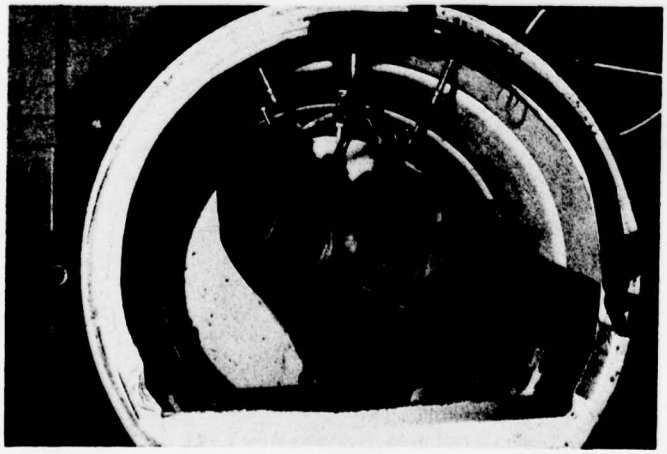
Figure 7. Restraint harness for HSG exposure of domestic fowl.



a. Centrifuge arm showing animal carriage, and slipring system (center) for signal transmission.



b. Animal carriage, which is mass loaded so that the bird remains oriented normally with respect to the acceleration field.



c. Bird in centrifuge with principal body axis 30° from "horizontal."

Figure 8. Installation of experimental animal in centrifuge carriage.

Score	Meaning
1	No apparent debilitation (normally ambulant, alert, aggressive)
2	Some minor debilitation (tendency to remain sitting, less alert, perhaps dazed)
3	Moderate debilitation (some specific disorder--such as opisthotonus, torticolus, postural instability)
4	Severe debilitation (multiple disorders--in the order of the previous category, but more extensive)
5	Moribund (limp, eyes closed, unresponsive; appearance such as to predict death)

Postmortem Examination

Animals dying or sacrificed for observation were subjected to a postmortem examination. The extent of this examination depended on the circumstances surrounding the animal's death.

Spontaneous deaths unrelated to the experimental protocol: The animals received a complete postmortem examination. All tissues were examined grossly, and portions of all major organ systems (with emphasis on those showing gross lesions) were sampled and processed for histologic examination. The objective of this procedure was to identify infectious disease and prevent any complications of experimental results with unrelated pathology.

Death or euthanasia following acute (single) exposure to HSG: All systems were examined for gross pathologic changes. The hearts and frequently the lungs were processed for routine histologic examination. In some cases, the hearts were weighed or were prepared for electron microscopy.

Death or euthanasia following chronic (recurrent) HSG exposure: In these birds all major organ systems were examined both grossly and histologically. When possible, the myocardium was perfused with fixative and prepared for ultrastructural examination.

In all birds the heart chambers were opened and washed free of excess blood. The endocardial surface was examined carefully; and the presence of either subendocardial hemorrhage (SEH), subendocardial congestion (SEC), or any other grossly evident change was noted.

Hematology

Evaluation of physiological responses to acceleration (including the development of systemic stress) frequently involved hematological evaluations: differential white-cell counts, PCV, and plasma proteins.

For the differentials, a drop of blood was collected by puncturing the wattle, and smears were prepared on cover slips by standard procedures (20,26).

These were air dried, fixed in absolute methanol, and stained by the Wrights-Leishman procedure. Prepared cover slips were mounted on slides for subsequent evaluation and storage.

For more extensive examinations, larger samples of blood were withdrawn from the brachial vein, using a heparin-rinsed syringe. PCVs were determined by the microhematocrit method--samples being centrifuged for 5 minutes at 11,000 RPM in heparinized capillary tubes. Plasma proteins were determined with a Goldberg Refractometer (handheld), with supernatant plasma from the hematocrit tube. (These devices measure the critical reflective angle, so the same result would be obtained with whole blood.)

Histologic Procedures

Tissue obtained for routine light microscopic examination was fixed immediately in 10% neutral buffered formalin, dehydrated through ascending concentrations of alcohol, embedded in paraffin, and sectioned at 5-6 μm. All sections were then stained with hematoxylin and eosin. Selected sections were stained with periodic acid-Schiff, phosphotungstic acid-hematoxylin, and Masson's trichrome. In animals selected for electron microscopic analysis, anesthesia was induced with a short-acting barbiturate, the thoracic cavity was opened, the brachiocephalic arteries were clamped, and the heart was perfused retrograde via the thoracic aorta with cold 2% glutaraldehyde. The endocardial surfaces were examined for the presence of subendocardial hemorrhage or congestion. Five blocks of myocardium were removed from each of the three left ventricular papillary muscles, dehydrated through ascending concentrations of alcohol, post-fixed in osmium tetroxide, and embedded in plastic (Epon 812). These blocks of tissue were sectioned at 1 μm, stained with toluidine blue, and examined by light microscopy. Areas within the blocks that showed an alteration in myocardial morphology were selected, trimmed, sectioned on an ultramicrotome, mounted on 200-mesh copper grids, stained with 50% lead citrate and saturated alcoholic uranyl acetate, and examined with an electron microscope.

ACCELERATION STUDIES

At the outset, some preliminary trials were required to develop a suitable restraint harness and also to estimate the approximate acceleration tolerance of the fowl. Once procedures were standardized, a research program was developed to achieve the contract objectives. The following discussion includes the principal investigative series of the program.

Screening Procedures

Once the repetitive treatment series were initiated, it became evident that a fairly large number of individuals were very susceptible and died after a few treatments. Since this complicated the establishment of long-term treatment series, we decided (in consultation with the technical monitors, 23 June 76) to subject all available animals to a standard treatment that would not be pathogenic but would cull susceptibles. Three acceleration schedules were established, as approximate to the tolerance of the various breeds and ages of the groups screened:

High intensity: 2, 3, or 4 minutes at 8 G. Applied to young Rhode Island Reds and some White Leghorn birds.

Intermediate intensity: 4, 8, or 12 minutes at 6 G. The standard procedure for skeletally mature Rhode Island Reds and some White Leghorn birds.

Low intensity: 4, 5, 8, 12, or 20 minutes at 4 G. Applied to older Rhode Island Reds (4, 6, or 8 minutes) or younger ones (8, 12, or 20) to compare group tolerances.

Large Breed (Rhode Island Red)

Results of the screening trials are included in Appendix A, and summaries of the tolerance distribution are presented in Table 1. Although these represent groups of animals raised and treated at different times, the percentage distribution of tolerance (for a particular breed at equivalent size) is remarkably uniform. This indicates that the wide variation seen between acceleration tolerance of otherwise equivalent individuals is inherent; so, in a genetically uniform stock, the occurrence of susceptibility or tolerance is statistical, and consequently rather uniform among large groups.

Postmortem examinations were made on birds not surviving the screening procedure, and a summary of the results is provided in Table 2. The numbers of birds involved are small, so no statistical confidence is attached to these results. However, rather systematically, a greater relative incidence of gross heart lesions occurred in the larger (and older) birds; also, fewer of these birds had no visible lesions (NVL). For example, compare the following distribution of lesions for all birds dying during screening, irrespective of their acceleration susceptibility:

	76 days old 1.11 ± 0.12 kg		127 days old 2.22 ± 0.18 kg	
	(n)	%	(n)	%
SEH	(22)	44.9	(26)	68.4
SEC	(9)	18.4	(10)	26.3
NVL	(18)	36.7	(2)	5.3

Small Breed (White Leghorn)

Groups of Leghorn chickens also were subjected to the screening procedures to compare the effects of mature body size upon the distribution of acceleration tolerance. Results of these screening trials are included in Appendix A, and a summary is presented in Table 3.

A comparison of the relative susceptibility of the two breeds indicates a much greater tolerance of the smaller breed, the Leghorns. This difference in response supports our selection of the larger bird for experimental subjects, since the object of the investigation is to examine the induction and nature of myocardial pathology.

TABLE 1. ACCELERATION TOLERANCE DISTRIBUTION[a] AMONG RHODE ISLAND RED MALES (LARGE BREED)

Days old =	289	83	76	127
Low Intensity (4 G):				
No. of birds in group			(30)	(30)
Body mass (kg) ± SD			1.28±0.18	2.34±0.24
Tolerance Category				
Low (<4 min)			0.0%	
(4-6 min)			0.0%	
(6-8 min)			3.3% } 9.7%	} 3.3%
Medium (8-10 min)			3.2%	
(10-12 min)			3.2%	
(12-20 min)			6.2%	3.3%
High (>20 min)			84.1%	93.4%
Intermediate Intensity (6 G):				
No. of birds in group	(53)	(183)	(60)	(30)
Body mass (kg) ± SD	3.14±0.28	1.24±0.23	1.11±0.12	2.22±0.18
Tolerance Category				
Low (<4 min)	22.6%	8.8%	8.3%	33.3%
Medium (4-8 min)	34.0%	19.8%	23.3%	26.7%
High (>8 min)	43.4%	71.4%	68.4%	40.0%
High Intensity (8 G):				
No. of birds in group			(60)	(30)
Body mass (kg) ± SD			1.13±0.13	2.37±0.23
Tolerance Category				
Very low (<2 min)			3.3%	23.3%
Low (2-3 min)			15.0% } 41.6%	6.7% } 60.0%
Medium (3-4 min)			23.3%	30.0%
High (>4 min)			58.4%	40.0%

[a]Screening-trial results were calculated to indicate distribution of acceleration tolerance by percentage of mortality at specific intensities and time intervals.

TABLE 2. GROSS PATHOLOGY OF RIR MALE BIRDS DYING DURING SCREENING

Tolerance category	Observed lesion	Rhode Island Red Males[a] 76 days old 1.11 ± 0.12 kg (n)	% [b]	127 days old 2.22 ± 0.18 kg (n)	% [b]
SCREENED AT 4 G:		(30)		(30)	
Low, <12 min		[3; 10% of group]		[1; 3% of group]	
	SEH	(0)	---	(0)	---
	SEC	(1)	33%	(1)	100%
	NVL	(2)	67%	(0)	---
Medium, 12-20 min		[2; 6% of group]		[1; 3% of group]	
	SEH	(1)	50%	(1)	100%
	SEC	(0)	---	(0)	---
	NVL	(1)	50%	(0)	---
SCREENED AT 6 G:		(60)		(30)	
Low, <4 min		[5; 8% of group]		[10; 33% of group]	
	SEH	(2)	40%	(7)	70%
	SEC	(1)	20%	(3)	30%
	NVL	(2)	40%	(0)	---
Medium, 4-8 min		[14; 23% of group]		[8; 27% of group]	
	SEH	(7)	50%	(7)	88%
	SEC	(1)	7%	(1)	12%
	NVL	(6)	43%	(0)	---
SCREENED AT 8 G:		(60)		(30)	
Very low, <2 min		[2; 3% of group]		[7; 23% of group]	
	SEH	(0)	---	(3)	43%
	SEC	(1)	50%	(3)	43%
	NVL	(1)	50%	(1)	14%
Low, 2-3 min		[9; 15% of group]		[2; 7% of group]	
	SEH	(4)	45%	(2)	100%
	SEC	(3)	33%	(0)	---
	NVL	(2)	22%	(0)	---
Medium, 3-4 min		[14; 23% of group]		[9; 30% of group]	
	SEH	(8)	57%	(6)	67%
	SEC	(2)	14%	(2)	22%
	NVL	(4)	29%	(1)	11%

[a]Experimental group hatched 15 February 1977.
[b]% of nonsurvivors at respective exposure.

TABLE 3. ACCELERATION TOLERANCE DISTRIBUTION[a] AMONG WHITE LEGHORN MALES (SMALL BREED)

Days old =	96	223	165	465
Intermediate Intensity (6 G):				
No. of birds in group	(36)	(30)	(65)	(36)
Body mass (kg) ± SD	1.12±0.14	1.80±0.31	2.04±0.24	2.22±0.30
Tolerance Category				
Low (<4 min)	0.0%	0.0%	1.5%	0.0%
Medium (4-8 min)	2.8%	3.3%	4.6	8.3%
High (8-12 min)	0.0%	0.0%	4.6%	8.4%
Very high (>12 min)	97.2%	96.7%	89.3%	83.3%

Days old =	146	223	178
High Intensity (8 G):			
No. of birds in group	(34)	(36)	(53)
Body mass (kg) ± SD	1.62±0.23	1.79±0.19	2.06±0.25
Tolerance Category			
Low (<2 min)	} 2.9%	0.0	} 22.6%
Medium (2-4 min)		25.0%	
High (>4 min)	97.1%	75.0%	75.0%

[a]Screening-trial results were calculated to indicate distribution of acceleration tolerance by percentage of mortality at specific intensities and time intervals.

Tolerance to Physiological End Points

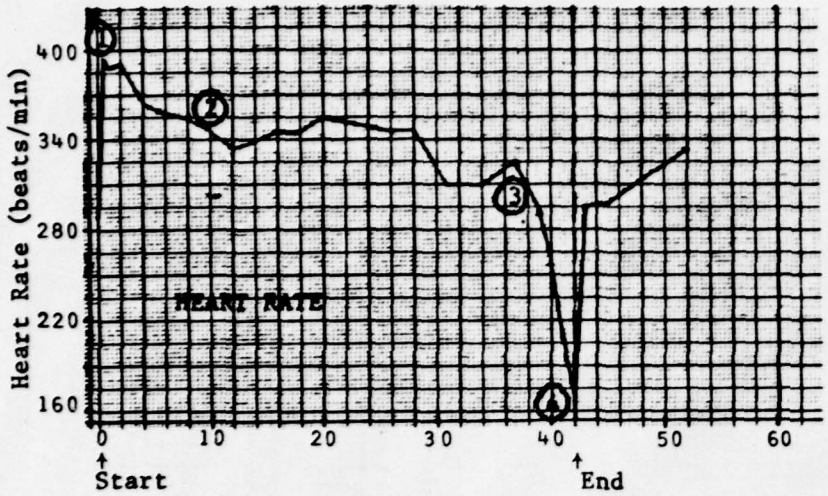
The acceleration-tolerance estimates obtained from the screening were limited to identifying only the most susceptible individuals in a group--those not surviving a particular acceleration exposure. Determining tolerance to a physiological endpoint is preferable since it provides a broader evaluation, with a tolerance index for each individual. One such estimate has been based on heart rate--specifically, the acceleration exposure compatible with a reasonably normal heart rate, which ordinarily is delineated by a rapidly developing bradycardia. We are pleased to acknowledge the collaboration of Dr. Jack Goldberg in establishing these procedures, and his comments on the bird EKGs are contained in Appendix B.

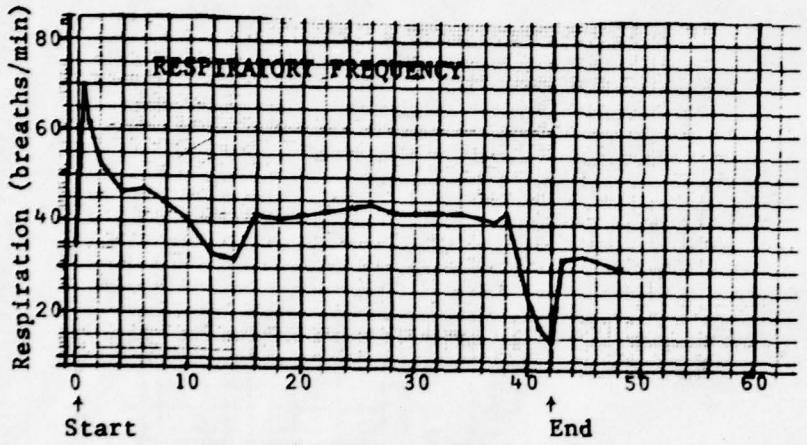
A system of sliprings on the centrifuge axis permits transmitting EKG signals from the operating centrifuge. EKG potentials were taken from subdermal electrodes, placed along the spine, to limit myoelectrographic interference. The electrocardiogram was recorded with a Type R Dynograph (Fig. 9). These were abstracted for heart rate and respiratory frequency (from the artifact it induces in the EKG). Several rather typical examples of such data are presented in Figure 10. In most cases, the heart and respiratory frequencies became stabilized after a transient period of increase or decrease. After some period, however, heart and respiratory frequencies started an abrupt decline; if centrifugation was not terminated immediately, the animal died. This bradycardia was taken as the animal's limit of acceleration tolerance.

Of the 61 RIR male birds examined, 43 survived treatment. The observed results and individual characteristics of the experimental birds are included as Appendix C. This experimental series developed over a long time. Birds were fitted in a few at a time between the repetitive treatment groups, and as a result, the group is diverse. Body mass (Fig. 11) has a trimodal distribution; and acceleration tolerance (minutes exposure to bradycardia, Fig. 12), a markedly skewed bimodal distribution. By comparison, the precentrifugation heart rate (Fig. 13) and its immediate response to centrifugation (Δ% heart rate over the first 20-sec to 1-min exposure, Fig. 14) present rather normal distribution patterns.

When these variables are summarized according to animal age at treatment and by the result of the treatment--survival or nonsurvival--some interesting relationships appear (Table 4). As the animals become older, they have less HSG tolerance and also develop a more pronounced bradycardia with the onset of acceleration. The nonsurvivors tend to be larger birds (+16% BM, $p < 0.02$) and with a faster pretreatment respiratory frequency (+44% RR, $p < 0.05$) than the survivors. The nonsurvivors also develop a more pronounced bradycardia with the onset of acceleration (-10% ΔHR as compared to -4% for survivors) and a slow breathing rate (-20% ΔRR), whereas the survivors develop a hyperpnea (+39% ΔRR); but these acceleration responses are quite variable and not statistically significant.

Linear regressions for several of the experimental parameters are summarized in Table 5. The tolerance to HSG is significantly and inversely correlated with body size and age. The size effect, however, is most pronounced in the younger animals. In the older birds (335±10 days) there was a nonsignificant but positive covariance between body size and tolerance. HSG tolerance is also significantly and positively related to pretreatment heart rate (HR), and this relationship remains more or less the same among the several age groups.





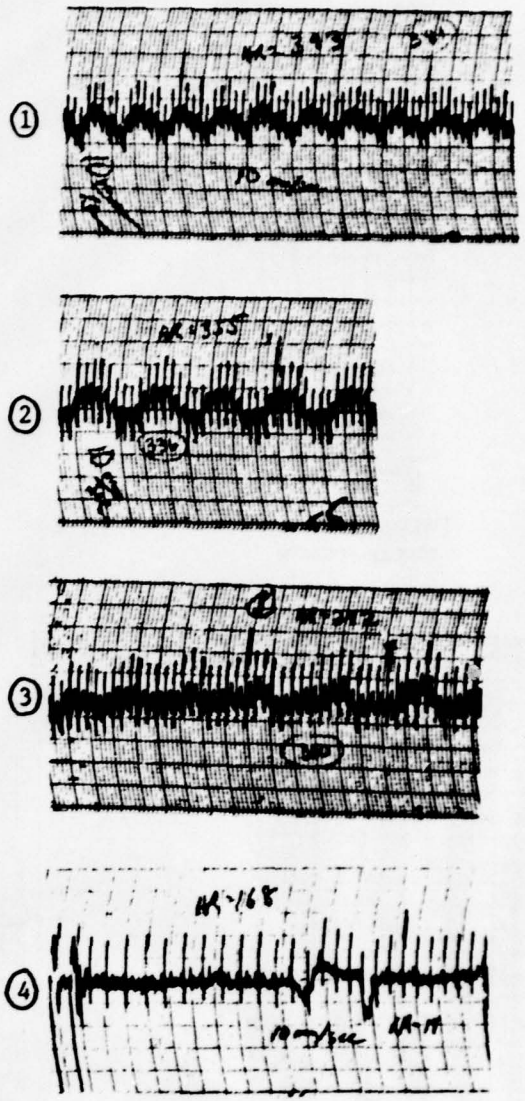


Figure 9. Cardiorespiratory function of RIR male bird (#2845) during exposure to +6 G_z. Numbered strips on the right are segments of the EKG tracing, from which the chart was constructed.

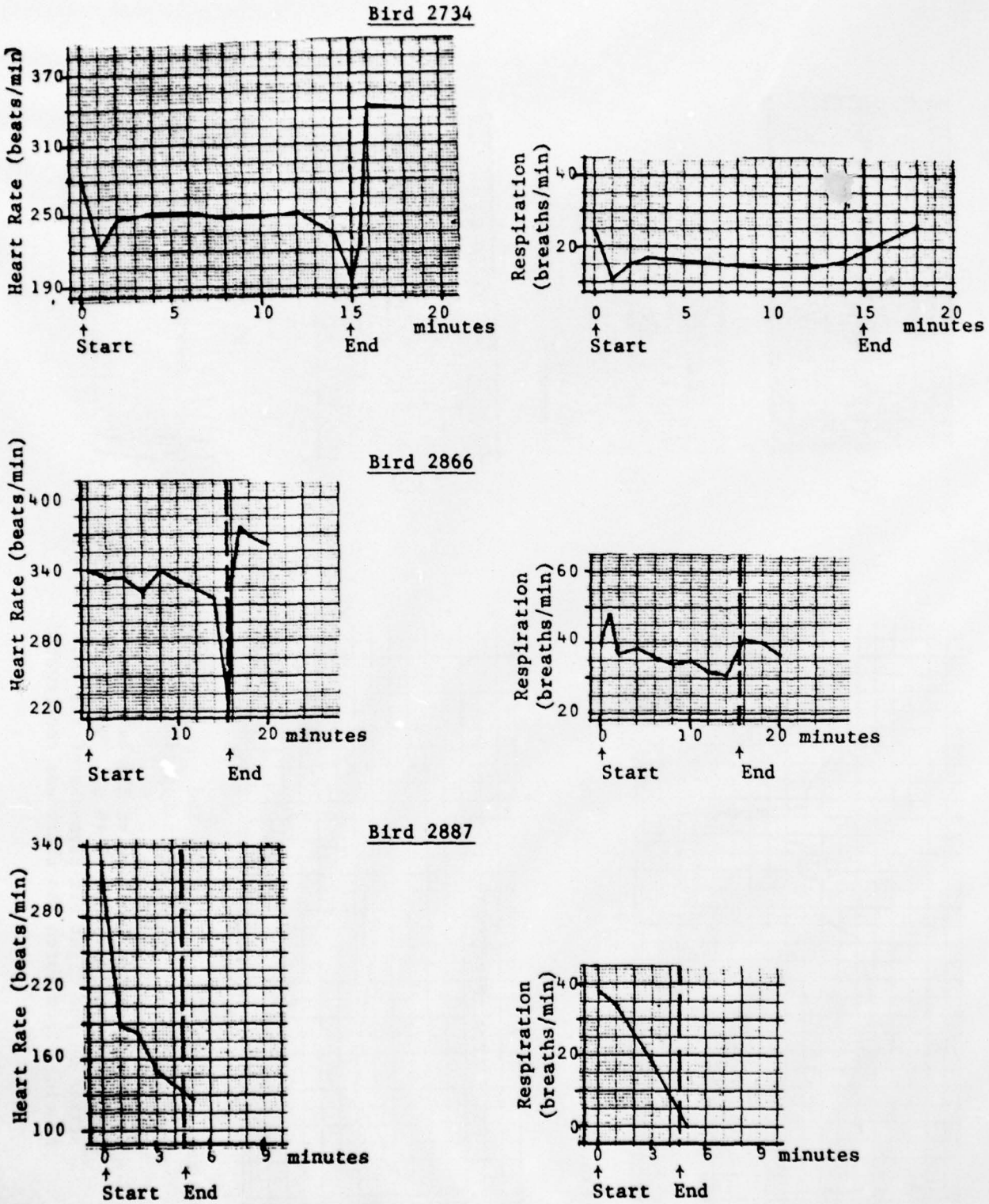


Figure 10. Cardiorespiratory function of RIR male birds exposed to +6 G_z. Period of centrifugation is indicated by arrows marked start and end.

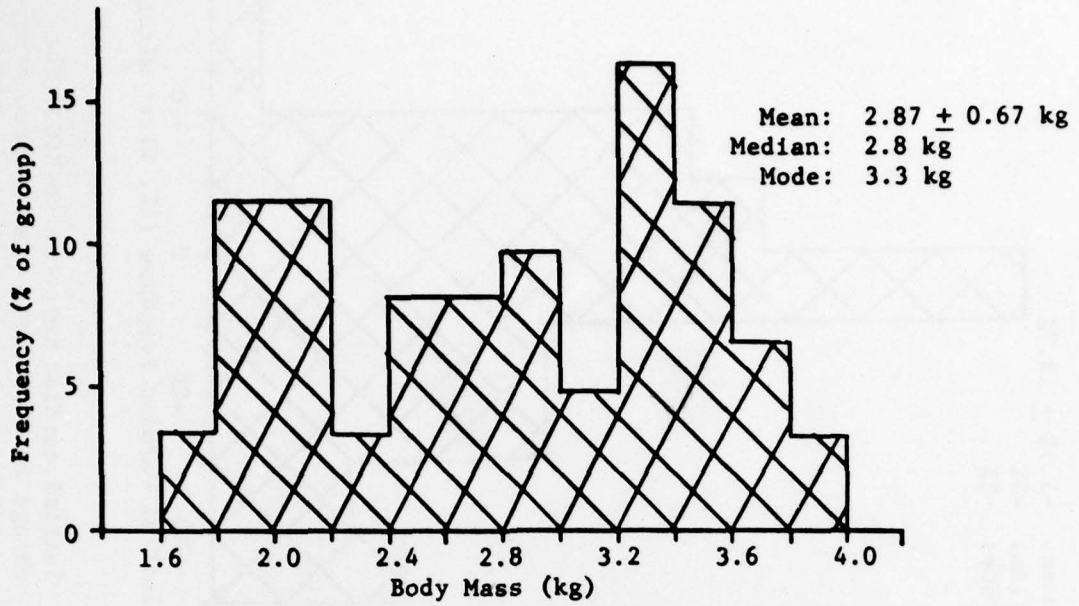


Figure 11. Distribution of body mass. In spite of its irregularity, distribution is fairly symmetrical, the mean and median being similar. Coefficient of variability [(SD/X) x 100], 23%, is not unusually high.

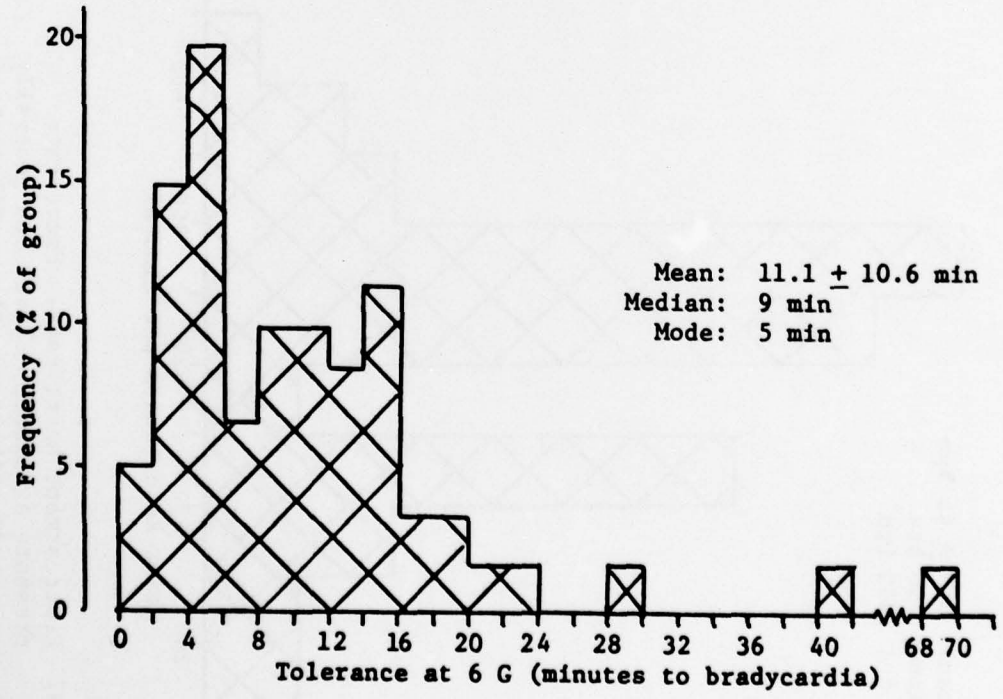


Figure 12. Distribution of HSG tolerance. Distribution is asymmetric, with marked differences between mean, median, and mode. Coefficient of variability is 95%.

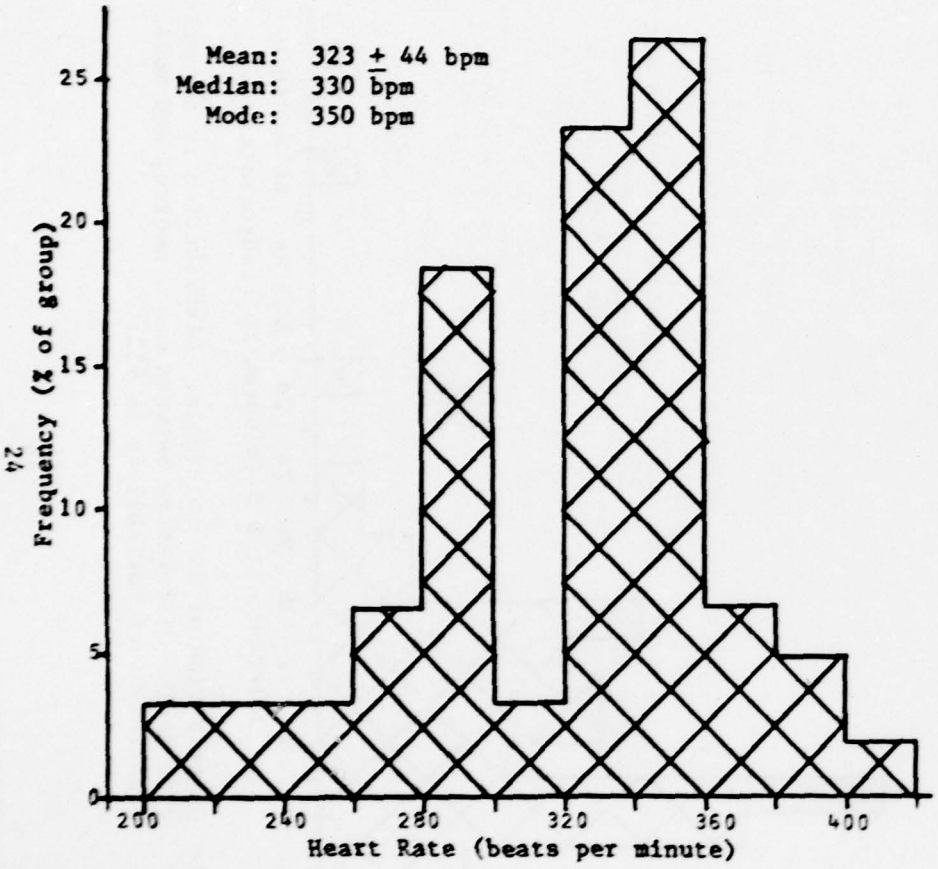


Figure 13. Pretreatment heart rate. Heart rate presents a slightly skewed but generally normal distribution. The coefficient of variation is 14%.

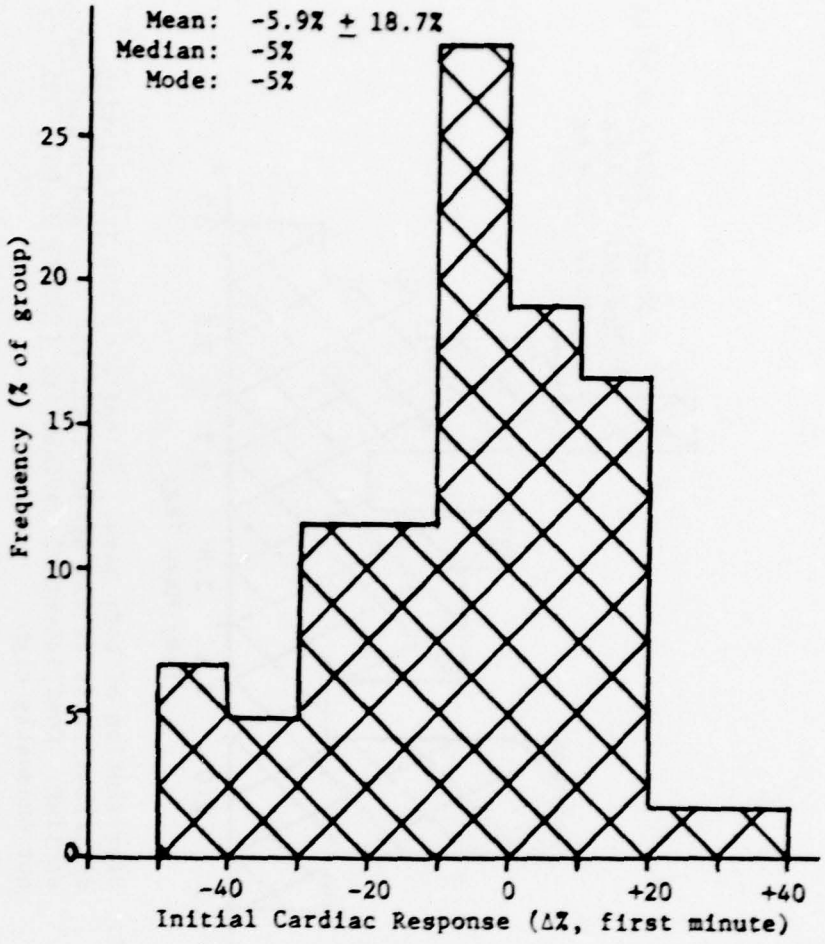


Figure 14. Initial cardiac response to HSG. The change in heart rate was measured over the first minute of centrifugation. This response was quite variable; 38% had a tachycardia, and the rest developed a bradycardia.

TABLE 4. HSG TOLERANCE AND RELATED FACTORS[a]

	(n)	Age (days)	Body mass (kg)	Tolerance (min)	Heart rate (bpm)	Δ% Heart rate
Age groups	(25)	122±16	2.20±0.34	13.7±13.2	348±34	-10.9±11.3
	(23)	259±10	3.36±0.35	11.0± 9.0	293±41	-8.7±24.0
	(10)	335± 1	3.17±0.40	6.8± 3.5	334±15	-12.1±16.4
	(3)	615± 0	3.42±0.14	3.7± 1.3	302±65	-22.3± 5.1
Survivors	(43)	216±104	2.74±0.63	12.3±12.1	326±44	-4.2±16.2
Nonsurvivors	(18)	274±148	3.19±0.67	8.3± 4.7	315±44	-9.9±23.8
ALL	(61)	233±120	2.87±0.67	11.1±10.6	323±44	-5.9±18.7

In two subgroups, information was obtained, alternatively, on respiratory frequency or on heart size and gross heart pathology. These are treated separately since they are not equivalent to the above groups or to each other.

	(n)	Age (days)	Body mass (kg)	Tolerance (min)	Respiratory frequency	Δ% Respiratory frequency
Age groups	(5)	146±11	2.22±0.44	25.1±25.4	26.2± 4.2	+76.6± 69.1
	(20)	259±10	3.39±0.35	11.3± 9.5	32.9±12.1	+11.1± 81.0
Survivors	(18)	225±51	3.03±0.63	17.0±16.1	28.2± 7.7	+41.3± 69.5
Nonsurvivors	(7)	265±10	3.50±0.35	6.4± 3.6	40.1±14.8	-19.7±100.2
ALL	(25)	236±47	3.16±0.60	14.1±14.5	31.6±11.3	+24.2± 81.9

	(n)	Age (days)	Body mass (kg)	Tolerance (min)	Relative heart size (g/kg)
Age groups	(20)	116±10	2.19±0.32	10.8±6.3	5.2±0.57
	(10)	335± 1	3.17±0.40	6.8±3.4	5.4±0.61
	(3)	615± 0	3.42±0.14	3.6±1.3	5.4±0.17
Survivors	(23)	205±136	2.47±0.52	8.5±6.1	5.2±0.56
Nonsurvivors	(10)	281±203	2.90±0.73	10.0±5.0	5.5±0.52
ALL	(33)	228±160	2.60±0.61	8.9±5.8	5.3±0.57

[a]Summaries = mean ± SD.

TABLE 5. PARAMETRIC RELATIONSHIPS[a]

Tolerance Relationships	**(n)**		
Body size:			
Survivors	(43)	$Tol = 1.56\ e^{-0.22BM}$	[r = -0.152, ns]
Nonsurvivors	(18)	$Tol = 25.0\ e^{-0.39BM}$	[r = 0.470; p <0.05]
ALL	(61)	$Tol = 18.4\ e^{-0.29BM}$	[r = 0.232; p = 0.07, ns]
Heart rate:			
Survivors	(43)	$Tol = 2.20\ e^{0.0041HR}$	[r = 0.214, ns]
Nonsurvivors	(18)	$Tol = 1.25\ e^{0.0055HR}$	[r = 0.456; p <0.05]
ALL	(61)	$Tol = 1.69\ e^{0.0048HR}$	[r = 0.265; p = 0.04]
Age:			
Survivors	(43)	$Tol = 13.5\ e^{-0.064t}$	[r = -0.183, ns]
Nonsurvivors	(18)	$Tol = 12.2\ e^{-0.058t}$	[r = -0.519; p <0.02]
ALL	(61)	$Tol = 13.1\ e^{-0.062t}$	[r = -0.253; p <0.05]
Correlates of Body Size			
Heart size:			
Survivors	(23)	$HM = 4.76\ BM^{1.088}$	[r = 0.903; p <0.001]
Nonsurvivors	(10)	$HM = 6.53\ BM^{0.849}$	[r = 0.938; p <0.001]
ALL	(33)	$HM = 5.07\ BM^{0.903}$	[r = 0.911; p <0.001]
Heart rate:			
Survivors	(43)	HR = 424 -36BM	[r = -0.506; p <0.001]
Nonsurvivors	(18)	HR = 414 -31BM	[r = -0.477; p <0.001]
ALL	(61)	HR = 419 -33BM	[r = -0.503; p <0.001]
Respiratory frequency:			
ALL	(26)	RR = 12.7 + 5.8	[r = 0.293, ns]
Correlates with Age			
Tolerance and body mass:			
Young (122±16d)	(25)	$Tol = 99.3\ e^{-1.045BM}$	[r = 0.417; p <0.04]
Medium (259±10d)	(23)	$Tol = 21.0\ e^{-0.279BM}$	[r = 0.120, ns]
Old (335± 1d)	(10)	$Tol = 0.58\ e^{-0.732BM}$	[r = 0.455; p = 0.11, ns]
Tolerance and heart rate:			
Young (122±16d)	(25)	$Tol = 2.18\ e^{0.0044HR}$	[r = 0.184, ns]
Medium (259±10d)	(23)	$Tol = 0.83\ e^{0.0078HR}$	[r = 0.400; p = 0.08, ns]
Old (335± 1d)	(10)	$Tol = 0.68\ e^{0.0064HR}$	[r = 0.233, ns]
Heart rate and body mass:			
Young (122±16d)	(25)	HR = 459 -51BM	[r = -0.501, ns]
Medium (259±10d)	(23)	HR = 227 +20BM	[r = 0.169, ns]
Old (335± 1d)	(10)	HR = 348 -5BM	[r = -0.079, ns]
Respiratory frequency and body mass:			
Young (122±16d)	(5)	RR = 13.0 + 5.9BM	[r = 0.618, ns]
Medium (259±10d)	(21)	RR = 16.1 + 5.0BM	[r = 0.255, ns]
Other Relationships			
Respiratory frequency vs. heart rate:			
ALL	(26)	RR = 31.9 -0.0027HR	[r = -0.0109, ns]
Immediate cardiorespiratory response:			
ALL	(25)	$\Delta\%RR = 23.6 + 0.097\Delta\%HR$	[r = 0.027, ns]

[a]Various animal characteristics were compared with treatment results to identify any covariances. Symbols and units used:

(n): number of observations involved;
Tol: HSG tolerance in minutes--exposure to a field of G-strength to produce a bradycardia;
BM: body mass (kg);
HR: heart rate, beats per minute;
t: time factor in regressions, as months of age;
HM: heart mass (g); relative HM: g heart/kg body mass;
RR: respiratory frequency, breaths per minute;
$\Delta\%HR$; $\Delta\%RR$: acceleration-induced changes as % difference from pretreatment value.

No significant relationships were found among pretreatment cardiorespiratory functions or their responses to the onset of acceleration.

In one subgroup that was routinely sacrificed after each trial, information was collected on heart size and posttreatment gross pathology--as well as on body size, HSG tolerance, and heart rate. The observed relationship between body size (BM, kg) and heart size (HM, g) is somewhat larger than reported in the literature (3), and may reflect the added presence of cardiac lesions:

$$H = 5.07\ BM^{1.04} \quad \text{(herein)}$$
$$H = 5.88\ BM^{0.98} \quad (3)$$

In this subgroup, the median HSG tolerance was about 10 minutes, and about 1/3 of the group did not survive the treatment. When the observations are divided upon the basis of HSG tolerance and survivorship, some interrelationships become apparent (Table 6). Relatively larger hearts are associated with a longer HSG tolerance, and also with nonsurvival. A similar association is seen in the gross heart pathology: more lesions appear in those with greater HSG tolerance and in those not surviving the treatment. These findings suggest that geometric (i.e., heart size) and temporal (HSG exposure time) factors are as important as biological (tissue) factors in the induction of such heart lesions.

Influence of Chronic Acceleration

Our laboratory is concerned also with the responses of animals to long-term (months) exposure to moderate fields (2 to 4 G). This treatment simulates a change in gravity and permits an estimate of gravitational influences. Since the animals can adapt physiologically, achieving a new steady state which does not change with continued exposure (i.e., any induced changes are maintained chronically), this treatment is described as chronic acceleration. Several reviews of the general results of such treatment are available (24,28,32).

A particularly interesting influence of this treatment is its modification of vascular characteristics and circulatory response, which have been reported by others with rats (12) that had been exposed previously to a 3.2-G field for 4 weeks. Although these observations may not duplicate the on-centrifuge situation, they are important in demonstrating changes in the vasculature and vasomotor function as a result of centrifugation.

Pressure-flow relationships in the posterior portion of rats were measured by means of cannulation of the abdominal aorta posterior to the renal artery. Four resistance components were identified:

Basal resistance (R_b) from vascular geometry and blood viscosity.

Local resistance (R_l) from smooth muscle activity that was independent of sympathetic discharge.

Sympathetic resistance (R_s) from the influence of sympathetic discharge upon the vasomotor apparatus.

Myogenic resistance (R_m) = $R_l + R_s$

Total resistance (R_t) = $R_m + R_b$

TABLE 6. ENDURANCE AND SURVIVAL OF TREATMENT RELATIONSHIPS IN PRODUCTION OF HEART LESIONS

Tolerance group (min)	Result of Treatment: Survivors (n)	Survivors	Non-survivors (n)	Non-survivors	ALL (n)	ALL
HSG Tolerance [minutes to bradycardia, $\bar{X}$ ± SD]:						
<10	(15)	4.8±2.4	(4)	5.0±1.2	(19)	4.8±2.2
>10	(8)	15.5±4.5	(6)	13.4±3.3	(14)	14.6±4.0
ALL	(23)	8.5±6.1	(10)	10.0±5.0	(33)	9.0±5.8
Relative Heart Size [g heart per kg body mass, $\bar{X}$ ± SD]:						
<10	(15)	5.23±0.53	(4)	5.14±0.32	(19)	5.21±0.49
>10	(8)	5.07±0.65	(6)	5.92±0.35	(14)	5.44±0.68
ALL	(23)	5.17±0.56	(10)	5.61±0.51	(33)	5.31±0.58
Heart-Body Size Relationship [all highly significant, p <0.001]:						
<10	(15)	$H = 5.06BM^{1.03}$ [r = 0.892]	(4)	$H = 5.51BM^{0.86}$ [r = 0.980]	(19)	$H = 5.31BM^{0.98}$ [r = 0.920]
>10	(8)	$H = 4.51BM^{1.14}$ [r = 0.896]	(6)	$H = 5.78BM^{1.02}$ [r = 0.973]	(14)	$H = 4.60BM^{1.19}$ [r = 0.916]
ALL	(23)	$H = 4.76BM^{1.09}$ [r = 0.903]	(10)	$H = 6.53BM^{0.85}$ [r = 0.938]	(33)	$H = 5.07BM^{1.04}$ [r = 0.911]
Gross Heart Lesions (% incidence):						
<10 SEH	(15)	13.3%	(4)	25%	(19)	15.8%
<10 SEC		0.0%		50%		10.5%
<10 NVL		86.7%		25%		73.7%
>10 SEH	(8)	62.5%	(6)	100%	(14)	78.6%
>10 SEC		12.5%		0%		7.1%
>10 NVL		25.0%		0%		14.3%
ALL SEH	(23)	30.4%	(10)	70%	(33)	42.4%
ALL SEC		4.4%		20%		9.1%
ALL NVL		65.2%		10%		48.5%

On the basis of these measurements, the postacceleration basal resistance decreased 20%, and the myogenic resistance increased 92% ($p < 0.05$). Consequently, resistance in an enhanced acceleration field increases vasomotor function.

Baroreflexes, which involve principally precapillary vascular changes, were evaluated by measuring resistance at various blood pressures that were obtained by treating the previously centrifuged animals with acetylcholine and epinephrine. The results indicated that chronic acceleration enhanced the responsiveness of baroreceptors two- to threefold.

Chemoreceptor reflexes were evaluated by measuring changes in systemic pressure and peripheral resistance accompanying a 20-second period of hypoxia (tracheal occlusion). Systemic pressures were reduced 40%, and peripheral resistance reduced 14%--both changes being statistically significant. These results indicate that chemoreceptor reflexes are depressed by previous chronic acceleration.

In view of these chronic acceleration effects upon circulatory function, groups of experimental birds for the present program were prepared by several months exposure to 2.5 G, and then examined for their response to HSG.

Screening Procedure

The chronically accelerated animals were put through the screening procedures along with previously untreated hatchmates. When results are compared, the chronic acceleration apparently did not affect the distribution of HSG susceptibility:

	Previously untreated birds	Chronic-acceleration adapted
Low tolerance (<4 min @ 6 G)	22.6%	10.0%
Intermediate tolerance (<8 min, >4 min @ 6 G)	34.0%	50.0%
High tolerance (>8 min @ 6 G)	43.4%	40.0%

Tolerance to Physiological End Points

One group of RIR birds was adapted to 2.5 G (2-month exposure) prior to evaluation of their HSG tolerance (at 289 days of age). Exposure required to induce a bradycardia in these previously accelerated animals was measured at 6 and 8 G--the latter after a 3-day rest period. Summaries of their screening responses and their capability to maintain a normal heart rate at 6 G (the latter compared with responses of previously untreated hatchmates) are shown in Table 7. The chronically accelerated animals are smaller (a characteristic result of this treatment) and quite tolerant to acute acceleration--almost 75% greater than previously untreated animals to 6 G (although the difference is not statistically significant). Chronically accelerated animals also exhibit greater heart and respiratory frequency responses upon acute acceleration--factors which appear to enhance acceleration tolerance in untreated animals.

TABLE 7. HSG TOLERANCE AFTER CHRONIC ACCELERATION ADAPTATION[a]

Tolerance Screening at 6 G

Acceleration treatment (min/G)	Group characteristics (n)	Group characteristics Body mass (kg) (Mean±SD)	Survivors (n)	Survivors Body mass (kg) (Mean±SD)	Nonsurvivors (n)	Nonsurvivors Body mass (kg) (Mean±SD)
4/6	(10)	2.72±0.21	(9)	2.69±0.20	(1)	2.99
8/6	(9)	2.69±0.20	(5)	2.66±0.28	(4)	2.72±0.06

Physiological Tolerance Limit--to a Bradycardia

	Mean±SD	Δ% from previously untreated birds (Table 1)
At 6 G_{+z} (n = 5)		
Body mass (kg)	2.69±0.19	-17.0, p <0.001
Tolerance (min)	30.0±21.7	+72.9, ns
Initial heart rate (bpm)	294±17	-1.7, ns
Change in heart rate (Δ%)	11.2±10.7	+195, p <0.02
Initial respiratory frequency (bpm)	21.7±7.45	-24, ns
Change in respiratory frequency (Δ%)	38.0±39.7	+93, ns
		Δ% from 6 G (above)
At 8 G_{+z} (n = 4)		
Body mass (kg)	2.63±0.10	-2.2, ns
Tolerance (min)	10.00±4.06	-67, p ≈0.09, ns
Initial heart rate (bpm)	283±26	-3.7, ns
Change in heart rate (Δ%)	19.3±3.68	+72, ns
Initial respiratory frequency (bpm)	18.0±6.0	-17, ns
Change in respiratory frequency (Δ%)	186.3±203.5	increased 3.9-fold, ns

[a] 2-month exposure to 2.5 G

The tolerances at 6 and 8 G appear to be related, but only the exponential relationship is statistically significant:

Arithmetic: $Tol_{8G} = -1.73 + 0.66\ Tol_{6G}$ [r=0.850, p=0.07, ns]

Exponential: $Tol_{8G} = 3.4e^{0.022\ Tol_{6G}}$ [r=0.909, p <0.04]

where: Tol = minutes exposed to a field of G intensity until a bradycardia develops.

There also appears to be some covariance for the change in respiratory frequency, but not heart rate, between the two treatments. Neither, however, is statistically significant:

Δ heart rate (Δ%H):

$$\Delta\%H_{8G} = -9.68 + 0.55\ \Delta\%H_{6G} \quad [r = 0.247, \text{ns}]$$

Δ respiration (Δ%R):

$$\Delta\%R_{8G} = 33.3 + 3.0\ \Delta\%R_{6G} \quad [r = 0.770, p = 0.08, \text{ns}]$$

Pretreatment

Early in the repetitive treatment series, the initial response (i.e., early mortality) appeared to be affected by the time lapse since the previously applied screening procedure. For example, the 1-day group was started 1 or 2 weeks after the initial screening and suffered an 83% first-day mortality. The 1-month group, however, was started very soon after the initial screening and had only a 22% mortality on the first day. The previous acceleration apparently had improved the response to the later HSG. Since the pretreatment was of a low order, it was not considered to have an adaptive effect; however, it apparently had conditioned the response (in the usual physiological meaning of the term), perhaps by enhancing the acuity of the hemodynamic reflexes. Familiarization with the centrifugation treatment, limiting adverse emotional responses, also was considered to be a possible factor.

The value of priming exposures on acceleration tolerance was tested by pretreating groups of birds that would be used in the 6-month repetitive series. Three pretreatment schedules were used:

Schedule I - one 1-minute 4-G exposure daily
II - four 1-minute 4-G exposures daily
III - no preconditioning

These schedules were applied for 1 week immediately before starting the animals on the 6-month treatment period. After the first regular treatment period (i.e., after four 4-minute exposures at 6 G) the appearance scores (page 14) of these birds were recorded; also, 4 hours later, blood samples were taken for hematological examination (these results are summarized in Table 8). From the change in relative lymphocytes (as well as mortality, indicated by decreasing sample size), preconditioning is seen to have a very beneficial effect on tolerating repetitive HSG. The minimal pretreatment schedule (schedule I) appears to be superior. The hematological response of those on schedule II indicates that this preconditioning was physiologically stressful. Subsequently, both pretreatment schedules were applied over much longer periods to determine if these induced any physiological changes.

Schedule II -- 1 minute at 4 G, 4 times daily

A group of six birds was placed on this regime when they had a mean size of 1.40 ± 0.36 kg--about 2/3 of mature body size. At the end of an 11-week period, the centrifuged birds were significantly smaller than their controls. This lesser body size appeared to be associated with a loss of depot fat (Table 9)--only one control bird lacked a developed abdominal fat pad and only one centrifuged bird had a significant fat pad. Both small size and loss of depot

TABLE 8. COMPARISON OF LYMPHOCYTE FREQUENCIES[a] FOR PRECONDITIONED AND NON-PRECONDITIONED RIR COCKS

	Group I 1 minute at 4 G once daily[b]		Group II 1 minute at 4 G 4 times daily[b]		Group III no preconditioning	
	(n)	mean±SD	(n)	mean±SD	(n)	mean±SD
Initial observation, prior to start of acceleration schedule:	(12)	62.4±8.7%	(18)	60.2±10.7%	(18)	69.5±9.6%
After AM treatment (4 minutes at 6 G, 4 times daily) -- first day schedule:	(8)	51.8±18.0%	(14)	38.4±13.8%	(14)	42.6±16.4%
After AM treatment, first day of second week:	(5)	52.0±11.4%	(9)	40.8±9.0%	(10)	42.7±15.8%
After AM treatment, first day of third week:	(4)	67.2±7.1%	(9)	49.0±11.1%	(6)	54.2±8.3%

[a]% of WBC 4 hours after regular acceleration treatment (four 4-min 6-G exposures), which is given twice daily.

[b]Preconditioning schedule for 1 week prior to acceleration treatment.

TABLE 9. AUTOPSY RESULTS AFTER SCHEDULE II PRECONDITIONING[a]

Bird No.	Weight (kg)	Comments
6	1.34	Died after 6th week; anemic; enlarged proventriculus with internal tissue masses; heart muscle flabby; general anemic appearance to internal tissues and organs.
31	1.48	Right kidney small with multifocal hard yellow nodules about 3-mm diameter; heart, 7.5 g; 1+ SEH on wall of left ventricle.
81	1.96	Focal congestion and/or hemorrhage in right lung; heart, 10.0 g; no abdominal fat pad.
156	2.25	Focal congestion and hemorrhage in left lung: heart, 14.4 g; substantial abdominal fat pad (53 g).
179	2.11	Focal pulmonary or hemorrhage in right lung; heart, 15.0 g; no abdominal fat pad.
186	1.93	Focal hemorrhage in left lung; heart, 11.6 g; 2+ SEH in major papillary muscles of left ventricle; no abdominal fat pad.

[a]Six birds were centrifuged 1 minute at 4 G, 4 times daily for 11 weeks, then sacrificed to determine any induced pathology.

fat are commonly encountered with chronically accelerated animals. A variety of tissue changes were observed, including two hearts with subendocardial hemorrhages, and congestive or hemorrhagic changes in lungs of four birds.

In the 11th week of pretreatment schedule II, blood was taken from the centrifuging group and from an equivalent control group to determine any hematological effect of the cumulated treatment, of the last week of treatment, and of the last day of treatment. The results, summarized in Table 10, indicate that the first 10 weeks had little persisting hematological effect; none of the observed differences between centrifuged and control animals had statistical significance. The last week of treatment (with an overnight recovery) had a more pronounced effect, with a highly significant reduction in the packed cell volume (-6%). However, a single-treatment series (Table 10: 5th day 11th week) appeared to induce marked decreases in lymphocyte frequency (-19%) and plasma protein concentration (-22%), the latter being highly statistically significant ($p < 0.001$). The decreasing plasma proteins appear to represent a selective loss through the vasculature (rather than a hemodilution), since the packed cell volume (PCV) increased slightly.

TABLE 10. ORGAN AND HEMATOLOGICAL CHANGES IN SCHEDULE II PRECONDITIONING[a]

	Schedule II (mean±SD)	Controls (mean±SD)	Rel. difference $(\frac{C - SII}{C} \times 100)$	
Body mass (kg)	1.94± 0.30	2.54±0.35	-24%	p <0.02
Relative heart mass (g/kg)	5.94± 0.91	5.88±0.41	-41%	ns
Rel. abdominal fat pad (g/kg)	4.7 ± 4.7	7.9 ±7.8	+1%	ns
Blood taken before treatment on 1st day of 11th week (weekend rest):				
PCV (%)	44.5 ± 4.1	45.8 ±4.3	-2.8%	ns
Plasma protein (g%)	5.54± 0.18	5.22±0.43	+6.1%	ns
Lymphocytes (%WBC)	70.6 ±12.0	78.8 ±4.4	-10.4%	ns
Blood taken before and after treatment on 5th day of 11th week:				
Pretreatment		Δ% from 1st day 11th week[b]		
PCV (%)	41.8 ± 4.1	-6.1 ± 3.9%		p <0.01
Plasma protein (g%)	5.44± 0.35	-1.8 ± 6.8%		ns
Lymphocytes (%WBC)	69.4 ± 3.2	+0.5 ±16.7%		ns
Posttreatment		Δ% from 5th day 11th week[b]		
PCV (%)	43.9 ± 5.4	+2.6 ± 4.5%		ns
Plasma protein (g%)	4.30± 0.26	-22.6 ± 3.3%		p <0.001
Lymphocytes (%WBC)	57.0 ±11.7	-19.0 ±19.6%		p = 0.10, ns

[a]Centrifuged 1 minute at 4 G, 4 times daily for 11 weeks, then sacrificed to determine any induced pathology.

[b]Statistical mean of % differences for individual birds.

The various results indicate that schedule II does induce physiological changes. Consequently a similar long-term preconditioning series was carried out with the less severe schedule I.

Schedule I -- 1 minute at 4 G, once daily

This group of six mature birds was centrifuged for 1 minute at 4 G once daily for a period of 10 weeks. No losses in body mass were noted, but there was a marked lack of abdominal fat pads (Table 11) as with schedule II. Each acceleration induced a small lymphopenia, which was highly significant at the first and sixth weeks. This was readily resolved between treatments, since no progressive lymphopenia was observed (Table 12).

TABLE 11. AUTOPSY RESULTS AFTER SCHEDULE I PRECONDITIONING[a]

Bird No.	Weight (kg)	Comments
32	3.03	5-mm cyst on kidney; heart, 22.7 g; small SEH at base of papillary muscle; small abdominal fat pad (2 g).
37	2.48	Heart, 23.1 g; no visible lesions; no abdominal fat pad.
44	2.57	Heart, 17.8 g; no visible lesions; no abdominal fat pad.
71	2.35	Small spleen; heart, 17.8 g; subendocardial congestion; no abdominal fat pad.
146	2.46	Small spleen; heart, 24.4 g; no visible lesions; no abdominal fat pad.
211	2.74	Small testes; heart, 19.7 g; no visible lesions; no abdominal fat pad.

[a]Six birds were centrifuged for 1 minute at 4 G once daily for 10 weeks, then sacrificed to determine any induced pathology.

TABLE 12. BODY MASS AND LYMPHOCYTES IN SCHEDULE I PRECONDITIONING[a]

		Lymphocyte frequencies[b]	
Week of treatment	Body mass[a] (kg ± SD)	% of WBCs pretreatment	4 hrs after treatment; ± Δ% of pretreatment[c]
1	2.60 ± 0.28	69.0 ± 9.5	-16.0 ± 11.3% p <0.01
2	2.55 ± 0.30		
3	2.55 ± 0.27		
4	2.58 ± 0.26		
5	2.57 ± 0.27		
6	2.56 ± 0.23	72.0 ± 9.5	-23.0 ± 7.2% p <0.001
7	2.57 ± 0.23		
8	2.58 ± 0.24		
9	2.60 ± 0.28		
10	2.60 ± 0.25	64.7 ± 7.8	
10	(5th day)	68.8 ± 8.9	-10.3 ± 14.2% p >0.10, ns

[a]Determined before the first treatment each week.
[b]Occasional pretreatment blood samples were taken for hematology; second blood sample also was taken 4 hours after the treatment.
[c]Statistical mean of % differences for individual birds.

This daily low-order acceleration schedule apparently does not induce physiological or pathological changes. Time, however, did not permit evaluating its effectiveness as a priming treatment--improving tolerance to a more severe schedule.

Repeated Treatment: 1 Day, 1 Week, 1 Month

So that suitable field intensities and exposure schedules could be determined, many birds were exposed over fairly short periods--in 1-day, 1-week, and 1-month groups--principally to identify bird survival and the induction of heart lesions. A summary of these groups is presented in Table 13.

TABLE 13. SUMMARY OF REPEATED-TREATMENT GROUPS (RIR MALES)

Groups (n=size)	Treatment (6 G)		Survive (%)	Pathology (%)		
	minutes	x daily		SEH	SEC	NVL
1-day groups						
(3)	1	8	100	33	0	67
(3)	2	8	67	33	0	67
(9)	3	8	56	83	0	17[a]
(8)	4	8	25	50	13	37
1-week groups						
(3)	1	8	100	33	0	67
(3)	2	8	100	33	0	67
(7)	4	8	71	20	0	80[b]
1-month groups						
(4)	1	8	75	33	0	67[b]
(3)	2	8	100	67	0	33
(9)	4	8	33	---	---	--[c]

[a] Postmortem observations for 2 (of 5) survivors.
[b] No postmortem observations for 2 nonsurvivors.
[c] No postmortem data.

The principal result of these experiments was establishing a treatment considered suitable for the 6-month repeated-treatment series--4 minutes at 6 G, 8 times daily. This series was effective in producing gross cardiac lesions; also, a reasonably high number of experimental animals could be expected to tolerate the regime for 6 months.

Repeated Treatment: 6 Months

The major task of this program was to develop a group of animals that had been centrifuged repeatedly for a 6-month period. The treatment schedule was the same as for the shorter cumulated-effect studies--4 minutes at 6 G, 8 times daily for 5 days each week. Mortality from this treatment was large; from an initial group of 48 animals, three completed the series. Various observations made on the experimental animals will be described separately.

Mortality Rate

A semilogarithmic plot of percent survivors versus duration of treatment (Fig. 15) produces a complex curve, indicating that the mortality rate is controlled by multiple processes. The observed survivorship curve can be mathematically resolved into its component parts by successively subtracting the slowest reaction--identified by the rectilinearity of its semilogarithmic plot (18,27). With this procedure, three separate modes of mortality become apparent.

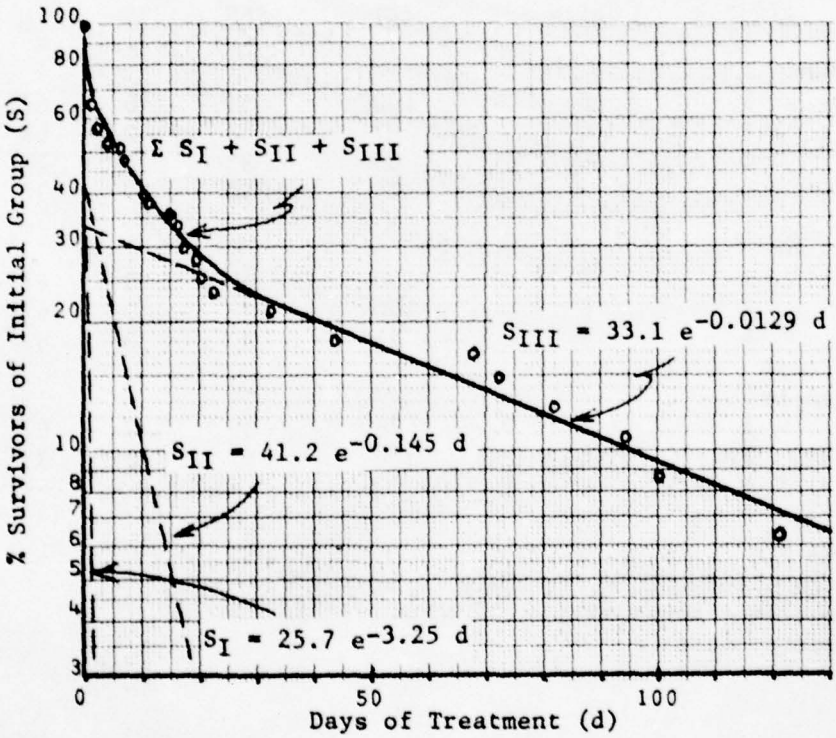


Figure 15. Mortality rate with repeated centrifugation. An analysis of survivorship curves indicates that there are three mortality modes--indicated as I, II, and III (in decreasing order of rate) on the chart. These modes appear to represent three subpopulations that exhibit different gross pathologies.

These have been extracted from the survivorship curve, and numbered (I, II, III) in the sequence of decreasing mortality rate. All are expressed as exponential relationships:

$$S_t = a\ e^{-kt}$$

where: S represents survivors as % of the initial complete group, and is S_t at time t (days);
a is the intercept value--the % of the initial group which dies by a particular mortality pattern;
k is the mortality rate, the fraction of the survivors dying per unit time, t.

Equations for the three mortality modes are:

$$S_{III} = 33.1e^{-0.0129d}$$

$$S_{II} = 41.2e^{-0.145d}$$

$$S_I = 25.7e^{-3.25d}$$

Mortality mode III appears to control the mortality rate at 20 days of treatment and beyond; up to day 19, mode II is dominant. From day 1 on, the sum of mode II and III survivors account for the total surviving group; but at day 0 (i.e., the intercept), approximately 25% of the initial group is not accounted for. This was interpreted as indicating a third mortality mode, which was peracute and not otherwise observed. Assuming that it accounted for 25.7% of the initial group, and that it ceased to control mortality rates after the first day, the cited equation (for I) was derived.

Occurrence of Acceleration Mortality

The nature of the treatment's interaction with the organism also appears to vary among the mortality modes. Mode I mortality (35% of the entire group) necessarily appears on Monday, the first day of acceleration. Mode II mortality occurs mostly on midweek days, and mode III mortality is distributed largely statistically:

Mortality (n)	Mon.	Tues.	Wed.	Thurs.	Fri.
I (17)	100.0%	--	--	--	--
II (18)	11.1%	44.4%	38.9%	--	5.6%
III (10)	30.0%	20.0%	30.0%	--	20.0%

The lethal process in acute acceleration appears to be quite abrupt, and is not anticipated. Mr. Kinder (who operated the centrifuge for this entire group) says that animal death in the centrifuge is always a surprise. This is in contrast to chronic-acceleration mortality, where all deaths (except peracutes) are preceded by a lymphopenia--or a perceptible change in appearance (9,10). The lack of change in outward appearance or behavior prior to acute-acceleration death can be demonstrated by comparing the appearance score (page 15, which is routinely made after centrifugation) over the 5 days preceding

death with that after the first 4 days of treatment. Two groups of birds were thus examined--8 with mode II mortality and a mean treatment of 13.5 ± 5 days prior to death; and 6 with mode III mortality and a mean treatment of 71 ± 27 days prior to death:

Day	Mode II	Mode III
Treatment:		
1 AM	1.8 ±1.0	1.3 ±0.5
PM	1.6 ±1.1	1.5 ±0.8
2 AM	1.5 ±0.8	1.1 ±0.4
PM	1.9 ±0.8	2.0 ±1.3
3 AM	1.5 ±0.9	1.3 ±0.5
PM	1.9 ±0.8	1.8 ±0.8
4 AM	1.8 ±1.6	1.5 ±0.5
PM	1.6 ±1.2	2.0 ±0.6
Mean: 1-4 days	1.7 ±0.95	1.58±0.74
Pretreatment:		
-5 AM	1.5 ±0.8	1.1 ±0.4
PM	1.5 ±0.8	1.3 ±0.5
-3 AM	1.4 ±0.5	1.3 ±0.5
PM	1.6 ±1.1	1.5 ±0.5
-2 AM	1.8 ±1.0	1.1 ±0.4
PM	1.9 ±0.8	1.1 ±0.4
-1 AM	2.3 ±1.8	1.1 ±0.4
PM	1.6 ±0.7	1.1 ±0.4
Mean: -5-1 days	1.69±0.97	1.25±0.44

Hematology

Hematological observations were made at weekly intervals, and results are summarized in Table 14 according to mortality mode. Lymphopenias developed early, but no systematic differences are evident between the three mortality modes. In the longer surviving group (mode III), the time course of the lymphocyte frequency is complex (Fig. 16). A recovery from an early lymphopenia was followed by a more severe lymphopenia, and finally a significant lymphocythemia developed (18.8% above the initial value, $p < 0.001$). Although mortality was occurring throughout, no systematic lymphocytic differences are apparent between the survivors and the entire group (Table 14).

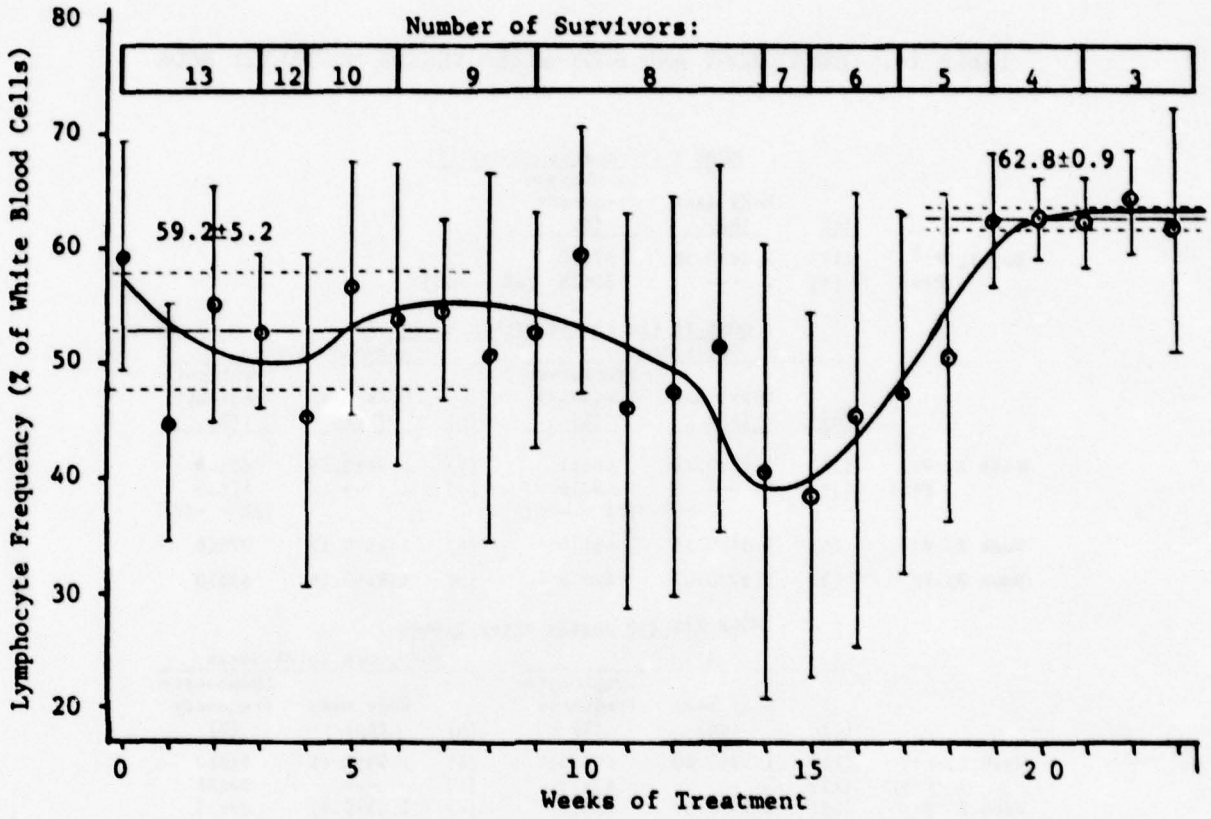


Figure 16. Lymphocyte frequency (mean ± SD), 6-month group (mode III mortality). (A similar plot for only those surviving to 20 weeks was not systematically or significantly different.) The irregular curve was drawn by inspection to indicate the general trend. Although a lymphopenia appeared to exist at 15 weeks, the final lymphocyte frequencies (62.8 ± 0.9 for the last 5 weeks) were significantly greater (+18.8%, $p < 0.001$) than the initial values (52.9 ± 5.2 for the first 8 weeks).

Postmortem Findings

Autopsies were conducted routinely on all animals not surviving the acceleration treatment. When the gross autopsy findings are compared with the mortality mode, the three mortality categories appear to have different pathogenic bases:

Mortality category	Period of dominance	% of initial group	Pathology (% of category sample) SEH	SEC	NVL
I	day 1	35.4	41.2	35.3	23.5
II	days 2-19	37.5	44.5	33.3	22.2
III	>day 19	27.1	15.4	7.7	76.9

TABLE 14. HEMATOLOGY AND BODY SIZE[a] VERSUS MORTALITY MODE

MODE I (17 deaths on day 1)

		(n)	Body mass (kg)	Lymphocyte frequency (%)	
Day 1,	P:[b]	(17)	2.39±0.38	67±10	
	P+4:	[5]	---	35±18	[Δ% = -48]

MODE II (18 deaths within 3 weeks)

		Survivors			Nonsurvivors		
		(n)	Body mass (kg)	Lymphocyte frequency (%)	(n)	Body mass (kg)	Lymphocyte frequency (%)
Week 1,	P:	(11)	1.61±0.19	66±11	(7)	1.94±0.59	65± 8
	P+4:	[11]	---	49±16 [Δ% = -26]	[7]	---	41±15 [Δ% = -37]
Week 2,	P:	(6)	1.56±0.16	48±10	(5)	1.45±0.17	37±18
Week 3,	P:	(3)	1.51±0.07	62± 7	(3)	1.81±0.14	43±10

MODE III (10 deaths after 3 weeks)

		All			Survivors to 24 weeks		
		(n)	Body mass (kg)	Lymphocyte frequency (%)	(n)	Body mass (kg)	Lymphocyte frequency (%)
Week 1,	P:[b]	(13)	1.83±0.40	59±10	(4)	1.93±0.49	51±10
	P+4:	[13]	---	42±13	[4]	---	34±11
Week 2	P:	(13)	1.79±0.39	45±10	(4)	1.89±0.47	39± 9
3	P:	(13)	1.86±0.32	55±10	(4)	2.05±0.26	52±10
4	P:	(13)	1.93±0.29	53± 7	(4)	2.03±0.34	54± 6
5	P:	(10)	1.93±0.24	45±14	(4)	2.02±0.37	55± 4
6	P:	(10)	1.96±0.19	57±11	(4)	2.05±0.30	57±16
7	P:	(10)	2.03±0.17	54±13	(4)	2.09±0.26	50±17
8	P:	(9)	2.03±0.19	55± 8	(4)	2.08±0.30	55± 8
9	P:	(9)	2.06±0.18	51±16	(4)	2.11±0.29	44±17
10	P:	(8)	2.09±0.17	53±10	(4)	2.11±0.32	53±10
11	P:	(8)	2.07±0.18	59±12	(4)	2.13±0.26	59± 9
12	P:	(8)	2.06±0.17	46±17	(4)	2.13±0.23	51±11
13	P:	(8)	2.08±0.18	47±17	(4)	2.16±0.22	55±13
14	P:	(8)	2.11±0.14	51±16	(4)	2.18±0.19	57± 7
15	P:	(8)	2.09±0.19	41±20	(4)	2.18±0.24	42±22
16	P:	(7)	2.14±0.18	39±16	(4)	2.17±0.27	44±19
17	P:	(6)	2.19±0.14	45±20	(4)	2.21±0.19	44±21
18	P:	(5)	2.22±0.17	48±16	(4)	2.23±0.21	44±18
19	P:	(5)	2.24±0.15	51±14	(4)	2.24±0.19	51±16
20	P:	(5)	2.24±0.16	63± 6	(4)	2.25±0.20	63± 6
	P+4:	[5]	---	48±10	[4]	---	47± 9
21	P:	(4)	2.26±0.19	62± 5	(4)	2.26±0.19	63± 3
	P+4:				[4]	---	47± 8
22	P:				(4)	2.27±0.18	63± 4
	P+4:				[4]	---	43±14
23	P:				(3)	2.24±0.19	64± 5
	P+4:				[3]	---	40±11
24	P:				(3)	2.14±0.23	62±11
	P+4:				[3]	---	44±18

[a]Mean ± SD.

[b]P--Prior to AM treatment; P+4--again 4 hours after AM treatment.

Microscopic analysis (described later) indicates that the animals not exhibiting gross pathology at autopsy are not necessarily unaffected by the treatment. Such animals appear to tolerate or perhaps compensate for the more fundamental histopathology.

Repeated Treatment: Modulated for Exposure Time or Intensity

When the results of the 6-month repeated-treatment experiments were evaluated, the very large mortality and significant lymphopenias indicated that systemic stress may have been the dominant factor (9,10). Since this may have altered or obscured myocardial pathologies, additional long-term treatment groups were established, with treatment schedules modulated so as to maintain relative lymphocyte frequencies within normal limits. Initial treatment levels were selected that would probably be tolerable, and these were either increased or decreased periodically to prevent systemic stress--with exposure time modulated for one group, and field intensity for the other:

Group designation	
Δ G	1 minute, 4 times daily; initially at 5 G, and increasing by 1-G increments at 7-week intervals.
Δ T	Exposed to 4 G, 4 times daily; initially for 2 minutes per exposure, and increasing by 1-minute increments at 7-week intervals.

The treatment schedules and their effect on body mass and relative lymphocyte frequencies are summarized in Tables 15 and 16. The means for the various modulated treatment periods (Table 17) indicate that neither group suffered the degree of lymphopenia encountered in the previous 6-month group. A mild lymphopenia (-10%) resulted from either field- or time-modulated treatment (about half as much as with the previous 6-month group), and this was readily resolved between treatments.

Autopsy findings also indicate that the modulated treatment schedule was the least severe for the 6-month group (Table 18). Gross heart lesions were seen only in animals dying before 30 days of treatment--roughly corresponding to mortality modes I and II of the previous 6-month series.

Maximum Field Tolerance Responses to More Intense but Briefer HSG Exposure

An important aspect of this investigation is the relationship between the intensity of acceleration and its duration, in the production of myocardial pathology. Most of the research relates to fields of 6 G--and with fairly prolonged exposures. A separate series was carried out, as the availability of animals and centrifuge schedules permitted, with the exposure fixed at 1 minute, but with field strengths generally increased in 1-G increments. The heart rate was monitored before, during, and afterwards, and lymphocyte frequencies were measured prior to and 4 hours after centrifugation. The individual results are presented in Appendix D, and summarized in Table 19.

TABLE 15. EFFECT OF MODULATED G-FIELD (ΔG) 6-MONTH TREATMENT[a] ON BODY MASS AND LYMPHOCYTE FREQUENCIES[b]

First Day of the Week				Last Day of the Week			
	Data prior to treatment		4 hours after		Data prior to treatment		4 hours after
Day of treatment	Body mass (kg)	Relative lymphocytes (% L)	treatment (% L)	Day of treatment	Body mass (kg)	Relative lymphocytes (% L)	treatment (% L)
(-21)		68±10					
(-14)		71± 8					
(-11)		72± 5					
(0)	2.87±0.34	71± 7					
			5 G				
				(3)	2.68±0.34	67± 6	53±15
(9)	2.56±0.41	65± 4		(13)	2.60±0.31	62± 8	54± 5
(19)	2.57±0.30	74± 9		(23)	2.55±0.29	63±10	60±16
(29)	2.46±0.09	63±13		(33)	2.48±0.09	66± 3	54± 8
(34)	2.48±0.09	66± 7					
			6 G				
(34)			51±10	(38)	2.40±0.12	65± 7	59± 9
(44)	2.41±0.14	66± 7		(48)	2.38±0.19	67±13	54± 7
(54)	2.37±0.20	68±10		(58)	2.35±0.20	69±13	62±10
(64)	2.33±0.22	65± 8		(68)	2.37±0.16	—	53±10
(70)	2.43±0.11	70± 3					
			7 G				
(70)			52±15	(73)	—	69± 7	57± 7
(79)	2.37±0.18	64± 9		(83)	2.38±0.11	69± 9	57± 5
(89)	2.41±0.09	63± 7		(93)	2.44±0.08	58± 7	54± 4
(98)	2.44±0.08	69± 8		(102)	2.42±0.07	63± 5	49± 5
(103)	2.42±0.07	66± 5					
			8 G				
(103)			55± 2	(107)	2.42±0.05	60± 7	51± 7
(113)	2.39±0.09	64±15		(117)	---	59± 5	49± 7

[a]Exposure for 1 minute, 4 times daily, with field strength controlled so as not to induce a relative lymphopenia.

[b]Mean ± SD.

TABLE 16. EFFECT OF MODULATED-EXPOSURE-TIME (ΔG) 6-MONTH TREATMENT[a] ON BODY MASS AND LYMPHOCYTE FREQUENCIES[b]

First Day of the Week				Last Day of the Week			
	Data prior to treatment		4 hours after treatment		Data prior to treatment		4 hours after treatment
Day of treatment	Body mass (kg)	Relative lymphocytes (% L)	(% L)	Day of treatment	Body mass (kg)	Relative lymphocytes (% L)	(% L)
(-21)		71±14					
(-14)		75± 9					
(-11)		72± 4					
(0)	2.32±0.24	78± 2					
2 minutes							
				(3)	2.24±0.23	64± 6	48±12
(9)	2.27±0.18	67± 3		(13)	2.16±0.20	67± 9	45±16
(19)	2.17±0.20	69±11		(23)	2.16±0.19	77± 6	56±27
(29)	2.18±0.19	72± 7		(33)	2.14±0.20	69± 1	46±24
(34)	2.14±0.20	62±21					
1.5 minutes							
(34)			48±23				
(35)	---	70± 7					
3 minutes							
				(38)	2.13±0.20	67± 4	53±28
(44)	2.12±0.23	74± 4		(48)	2.20±0.22	74± 8	66±14
(54)	2.26±0.12	73± 4		(58)	2.29±0.12	68± 3	68± 9
(64)	2.30±0.11	65± 8		(68)	2.26±0.10	68± 5	59±13
(70)	---	61± 3					
4 minutes							
(70)			59± 5	(73)	2.31±0.11	---	63± 8
(79)	2.33±0.12	70± 5		(83)	2.25±0.09	72± 2	63± 5
(89)	2.24±0.09	68± 7		(93)	2.23±0.11	73± 4	62± 8
(98)	2.24±0.11	---		(102)	2.23±0.11	73±11	70± 2
(103)	2.23±0.11	71± 8					
5 minutes							
(103)			57±16	(107)	2.23±0.16	69±12	57± 4
(113)	2.22±0.14	65± 6		(117)	---	---	61± 4

[a]Exposure to 4 G, 4 times daily, with exposure times varied (1.5-5 min/exposure) so as not to induce a relative lymphopenia.

[b]Mean ± SD.

TABLE 17. MODULATED (Δ G and Δ T) 6-MONTH TREATMENT

Period (days of treatment)	Field (G)	Survivors (n)	Body mass (kg)	Relative Lymphocytes (% WBC) First day (pre-)	Fifth day (pre-)	Fifth day 4 hours (post-)
		Field modulated -- Δ G				
Pretreatment ± SD	1	(6)	2.87 ±0.34	70.2 ±7.2	----	----
0-34	5	(5)	2.52	67.0	64.5	55.2
35-70	6	(4)	2.39	67.3	66.3	57.0
73-103	7	(4)	2.41	65.5	64.8	54.3
107-117	8	(4)	2.39	64.0	59.5	50.0
		Time modulated -- Δ T				
	4-G exposure (min)					
Pretreatment ± SD	0	(6)	2.32 ±0.24	74.0 ±7.8	----	----
0-34	2	(5)	2.18	67.5	69.3	48.8
35-70	3	(4)	2.23	68.3	69.3	61.5
73-103	4	(3)	2.26	69.3	73.8	64.5
107-117	5	(3)	2.22	65.0	68.5	59.0

TABLE 18. AUTOPSY FINDINGS--MODULATED (Δ G and Δ T) TREATMENT GROUPS

	Bird No.	Total days of treatment	Lesions
Δ G group	1	116 (sacrificed)	NVL
	9	2 (died)	SEH
	15	28 (died)	SEH
	64	116 (sacrificed)	NVL
	140	116 (sacrificed)	NVL
	190	116 (sacrificed)	NVL
Δ T group	69	116 (sacrificed)	NVL
	194	81 (died)	NVL
	199	1 (died)	SEH
	206	50 (died)	NVL
	227	116 (sacrificed)	NVL
	228	116 (sacrificed)	NVL

TABLE 19. PHYSIOLOGICAL RESPONSES[a] TO 1-MINUTE HSG EXPOSURE

Field (G)	(n)	Body size (kg)	Age (days)	Lymphocytes		Initial heart (bpm)	Change in Heart Rate (Δ%)				
				Initial (%)	4 hrs after (Δ%)		Centrifugation		Postcentrifugation		
							0-20 (sec)	0-60 (sec)	0-20 (sec)	0-60 (sec)	60-120 (sec)
5	(3)	2.71± 0.14	224	78± 5.3	-28.1± 10.7	281± 33.5	+15.0± 9.5	+1.7± 14.3	-5.3± 9.6	-5.0± 8.7	-8.3± 10.4
6[b]	(3)	2.57± 0.52	224	72± 9.6	---	309± 8.1	+4.7± 25.5	-7.7± 27.5	---	---	---
7	(3)	2.52± 0.42	224	67± 12.2	-23.9± 7.1	306± 18.3	+7.0± 5.6	-4.0± 4.6	-9.0± 3.0	-8.0± 4.4	-12.7± 5.5
8	(3)	1.80± 0.30	101	67± 5.6	-1.7± 9.9	351± 11.0	+6.0± 1.0	-3.0± 6.9	-11.7± 16.6	-2.3± 4.0	+1.0± 8.2
8	(6)	2.60± 0.05	203	67± 11.7	-38.3± 20.3	290± 27.2	+10.3± 14.7	-11.7± 20.1	-9.3± 21.9	+1.2± 10.6	+2.7± 8.2
8	(all 9)	2.33± 0.43	152	67± 9.7	-26.1± 24.8	310± 37.7	+8.9± 11.9	-8.8± 16.8	-10.1± 19.2	0.0± 8.8	+2.1± 7.7
9	(3)	1.64± 0.22	102	68± 6.6	-15.6± 19.9	329± 7.0	+11.0± 6.6	-3.7± 7.1	-0.7± 19.1	+0.7± 10.5	+6.3± 5.1
10	(3)	1.77± 0.28	102	74± 10.3	-35.6± 5.9	338± 35.6	+3.0± 4.0	0.0± 2.7	+3.7± 2.5	+7.0± 1.0	+5.5± 2.1
10	(3)	2.59± 0.20	141	76± 6.8	-55.6± 18.0	321± 31.2	+9.3± 2.1	-11.7± 15.1	-25.5± 30.4	+16.3± 15.0	+5.3± 9.3
10	(6)	3.13± 0.34	204	61± 16.9	-20.5± 25.0	269± 28.8	+3.0± 6.1	-21.8± 11.6	-36.5± 12.1	-13.0± 5.7	+2.5± 11.1
10	(all 12)	2.66± 0.64	149	68± 15.6	-36.6± 25.5	299± 42.8	+4.6± 5.4	-13.8± 13.9	-23.5± 22.2	-0.7± 15.3	+3.8± 9.0

[a]Mean ±SD. See Appendix D for individual data.
[b]Animals centrifuged for 1.5 minutes.

TABLE 19 (continued)

Field (G)	(n)	Body size (kg)	Age (days)	Lymphocytes Initial (%)	Lymphocytes 4 hrs after (Δ%)	Initial heart (bpm)	Change in Heart Rate (Δ%) Centrifugation 0-20 (sec)	Centrifugation 0-60 (sec)	Postcentrifugation 0-20 (sec)	Postcentrifugation 0-60 (sec)	Postcentrifugation 60-120 (sec)
11	(3)	1.51± 0.47	118	68± 2.5	-31.6± 17.9	331± 14.0	-1.3± 20.8	-10.0± 19.1	-0.7± 15.9	-0.3± 15.0	+0.3± 7.8
12	(3)	1.96± 0.43	118	75± 3.8	-22.0± 7.6	322± 8.1	+11.0± 11.1	-5.7± 23.0	-7.3± 36.6	-1.3± 23.5	-6.0± 15.0
12	(3)	2.19± 0.08	152	69± 6.0	-43.3± 6.0	290± 30.3	+14.0± 9.5	-20.3± 6.0	-12.7± 4.9	-8.0± 8.7	-7.7± 7.6
12	(all 6)	2.08± 0.31	135	71± 9.2	-32.3± 13.5	306± 26.2	+12.5± 9.4	-13.0± 17.0	-10.0± 23.5	-4.7± 16.2	-6.8± 10.7
13	(3)	2.20± 0.30	137	66± 14.9	-29.0± 19.6	349± 8.7	-2.0± 5.3	-15.0± 20.9	-15.7± 8.4	-7.0± 9.0	+2.0± 7.9
14	(6)	2.50± 0.20	153	66± 11.9	-29.0± 13.0	312± 10.2	-14.5± 22.5	-27.5± 27.7	-42.7± 20.0	-25.8± 19.9	-3.0± 14.8
15	(3)	2.36± 0.38	194	73± 8.5	-45.0± 30.6	279± 52.6	+8.7± 23.8	-26.3± 16.0	-41.7± 14.2	-13.7± 19.5	-10.0± 19.3
16	(3)	2.73± 0.20	195	62± 20.2	-48.6± 25.6	284± 38.1	-3.0± 13.1	-22.0± 16.1	-32.3± 14.1	-11.0± 13.5	-0.7± 15.7
17	(3)	3.00± 0.49	294	85± 3.5	-53.8± 13.3	290± 44.8	-14.3± 22.6	-34.0± 6.0	-44.0± 11.3	-25.3± 5.1	-7.0± 5.3
18	(3)	2.25± 0.19	140	81± 2.6	-38.6± 6.7	326± 5.0	-6.0± 3.5	-34.3± 1.5	-40.3± 9.0	-21.3± 12.4	+9.7 ± 6.4

A total of 63 birds were treated in this series, all of which survived. Several were sacrificed, and no evidence of gross heart pathology was noted. Apparently, then, chickens are quite tolerant to very high acceleration fields when the duration of exposure does not exceed 1 minute. A variety of changes, however, were observed in cardiac and vegetative function, indicating that the treatment had a physiological effect which generally was proportional in degree to field strength.

Heart Rate

The influence of the 1-minute HSG exposures upon heart rate (HR), as Δ% HR of pretreatment value, is shown for several time periods in Figures 17-22. Over the first 20 seconds in fields of 12 G or less, the birds generally exhibit a tachycardia; to more intense fields, the general response is a bradycardia (Fig. 17). Over this initial period the relationship of field strength upon heart rate appears to be arithmetic:

[20 seconds centrifugation]
$$\Delta\% \text{ HR} = 18.55 - 1.55\,(G-1) \quad [r = -0.693;\ p < 0.01]$$
$$= -1.55\,(G-12.97)$$

Over the full minute of centrifugation, the general effect is a bradycardia (Fig. 18), although apparently a tachycardia would be maintained in fields below 6 G. There also appears to be an interaction between the treatment and animal age (see Table 19), with older birds having a greater bradycardia in equivalent fields. The influence of field strength upon heart rate over the full minute of treatment appears to be arithmetic:

[1 minute centrifugation]
$$\Delta\% \text{ HR} = 11.34 - 2.57\,(G-1) \quad [r = -0.936;\ p < 0.001]$$
$$= -2.57\,(G-5.42)$$

With cessation of centrifugation, an intensification of the acceleration-induced bradycardia occurs--rather than a rebound tachycardia which may have been anticipated. This relationship is indicated in Figure 19, which compares heart rate for the first 20 seconds after centrifugation with field strength. Again, the kinetics appear to be arithmetic:

[20 seconds post centrifugation]
$$\Delta\% \text{ HR} = 16.10 - 3.42\,(G-1) \quad [r = -0.816;\ p < 0.001]$$
$$= -3.42\,(G-5.71)$$

The tendency for a more pronounced postcentrifugation bradycardia is transient, and diminishes over the entire first minute after centrifugation (Fig. 20):

[1 minute post centrifugation]
$$\Delta\% \text{ HR} = 9.26 - 1.71\,(G-1) \quad [r = -0.725;\ p < 0.01]$$
$$= -1.71\,(G-6.42)$$

The transient nature of the centrifugation bradycardia is also indicated by the direct comparison of postcentrifugation heart rate with the heart rate during centrifugation (Fig. 21):

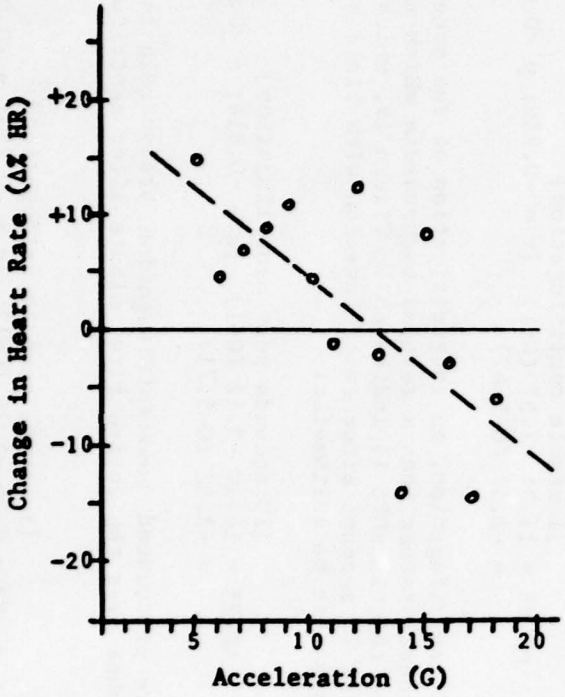


Figure 17. Mean cardiac response to first 20 seconds exposure to HSG.

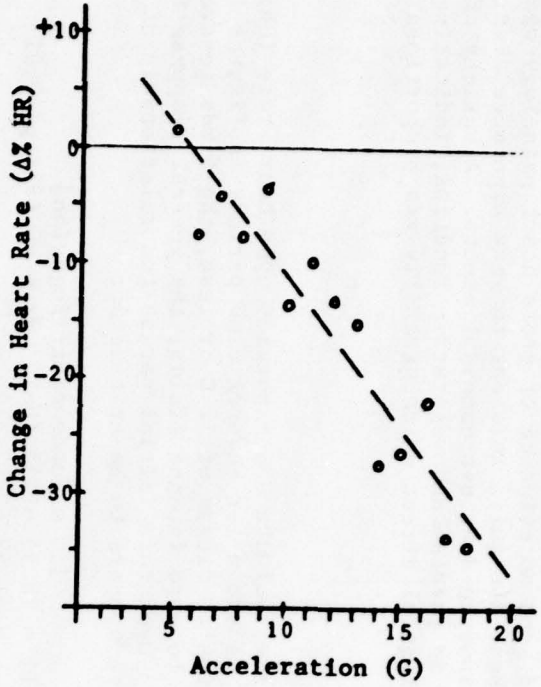


Figure 18. Mean cardiac response to 1 minute of HSG.

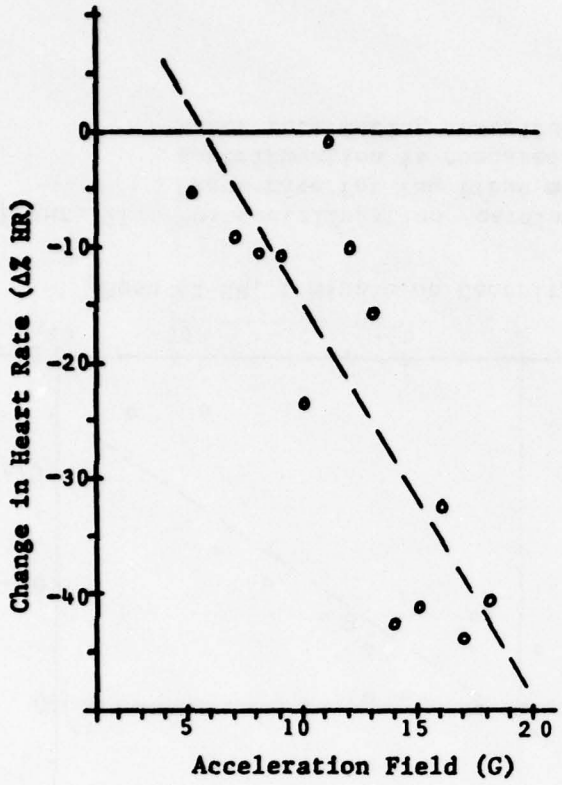


Figure 19. Immediate postcentrifugation (first 20 seconds) cardiac response. In this period, the slope of the heart rate versus G curve is approximately double the value for 1 minute of centrifugation (Fig. 18).

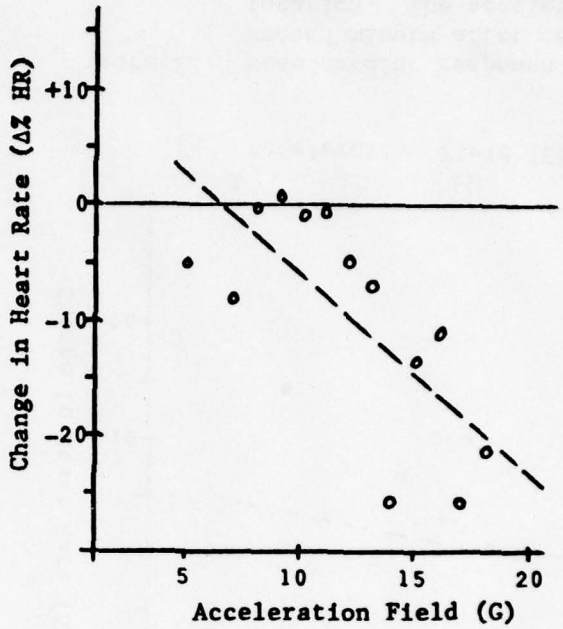


Figure 20. Mean cardiac response 1 minute post centrifugation. The marked bradycardia that accompanies release from an intense acceleration field is quickly ameliorated--and 1 minute after centrifugation, the heart rate depression is substantially less than during centrifugation.

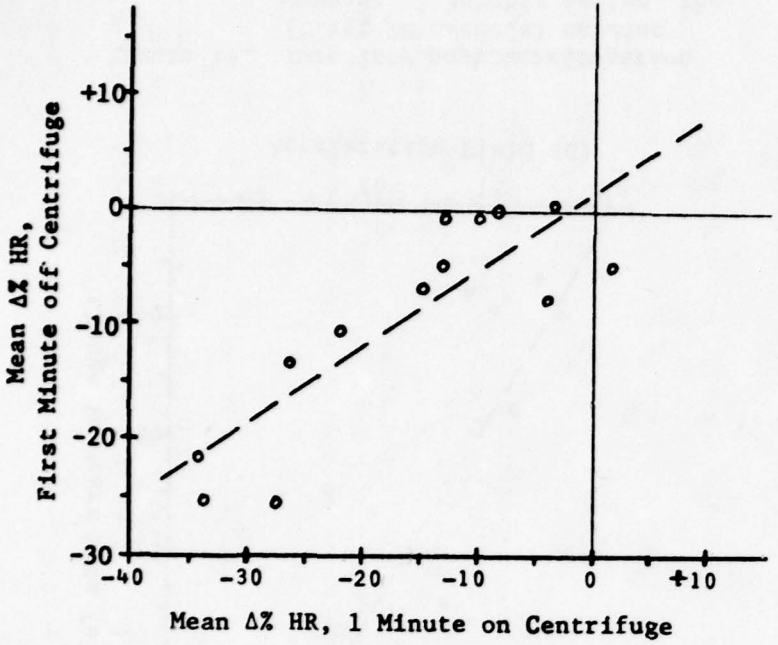


Figure 21. Postcentrifugation cardiac changes. Heart rate for the first minute after centrifugation is compared with the heart rate during centrifugation.

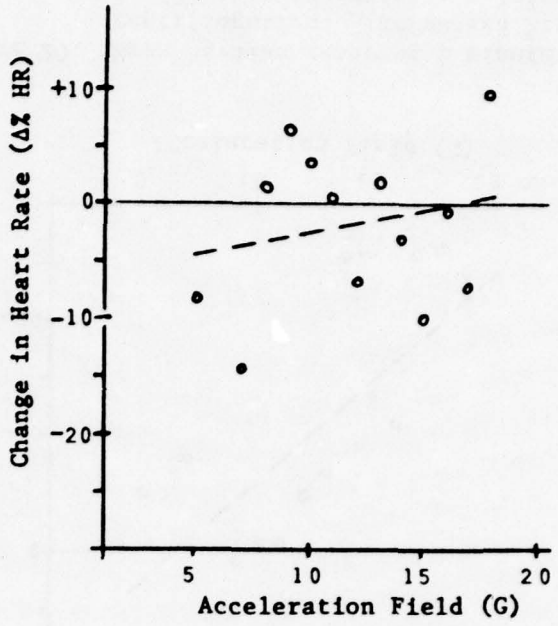


Figure 22. Mean cardiac response in second minute after centrifugation. The acceleration-induced bradycardia is largely gone. Half of the animals have a tachycardia, and the heart rate is no longer related to the previous treatment (HSG).

$$\Delta\% \text{ HR}_{off} = 0.68\ (\Delta\% \text{ HR}_{on} - 2.46)$$
$$[r = 0.842;\ p < 0.001]$$

During the second minute after centrifugation, about half of the animals have regained their precentrifugation heart rate, and there is no longer any evidence of a systematic influence of the previous treatment (Fig. 22). In the second minute after centrifugation, the heart rate is not significantly related to either the precentrifugation or the centrifugation heart rate:

$$\Delta\% \text{ HR} = -5.84 + 0.364\ (G-1) \quad [r = 0.111;\ ns]$$
$$= 0.364\ (G - 16.06)$$

Lymphocyte Frequency

Blood smears prepared from each bird before centrifugation and again 4 hours later were evaluated for lymphocyte frequency, to determine the presence of a systemic stress. Results of these studies are summarized in Figure 23; although there is much variability, the relative lymphocyte frequency [$\% L = (L/L_o) \times 100$] appears to be exponentially related to field strength:

$$\Delta\% L = 11.73 - 2.00\ (G-1) \quad [r = -0.756;\ p < 0.01]$$
$$= -2.0\ (G + 4.88)$$

A more reasonable relationship is obtained by forcing it through the origin--so that no lymphopenia is indicated for normal gravity:

$$\Delta\% L = -2.95\ (G-1)$$

These results indicate that at least a minute's exposure to a 2.5-G field is required to elicit a lymphopenia, and at least a 7- or 8-G field is required to elicit a significant lymphopenic response--a 25% decrease in lymphocyte frequency.

Body Mass

The birds were weighed periodically following the 1-minute exposure to HSG, and the results (BM as % of pretreatment BM) are summarized in Figure 24. A common response to this treatment was a 1- or 2-day period of growth repression, found rather uniformly up to a 13-G field. At this point, this growth response became persistent and lasted about 2 weeks. Above 13 G, growth repression was presumably the result of a metabolic response to the treatment. Above 16 G the effect became more pronounced, also more persistent.

Field (G)	(n)	1 day	3 days	6 days	10 days	14 days	20 days
5-13	(40)	99.7±2.2	100.1±2.4	101.8±3.5	104.3±5.4	107.0±7.0	---
14-15	(9)	98.9±2.2	98.6±2.7	98.9±3.9[a]	98.9±4.6[b]	100.0±4.7[c]	
16-18	(9)	99.1±2.5	97.2±4.2[a]	97.3±5.0[a]	96.5±6.4[b]	98.6±6.5[b]	99.5±6.0

[a] <0.05, [b] <0.01, and [c] <0.001--probabilities of difference from the 5- to 13-G group.

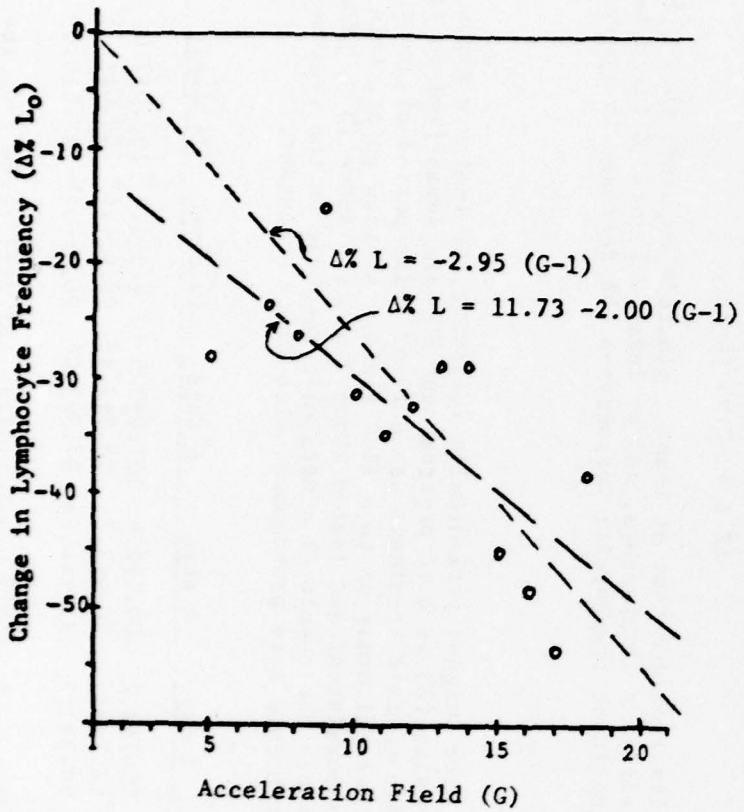


Figure 23. Lymphocyte response to HSG. Lymphocyte frequencies (L/100 WBCs) were measured before and 4 hours after centrifugation. In all cases a lymphopenia was observed--which appeared to be arithmetically related to field strength.

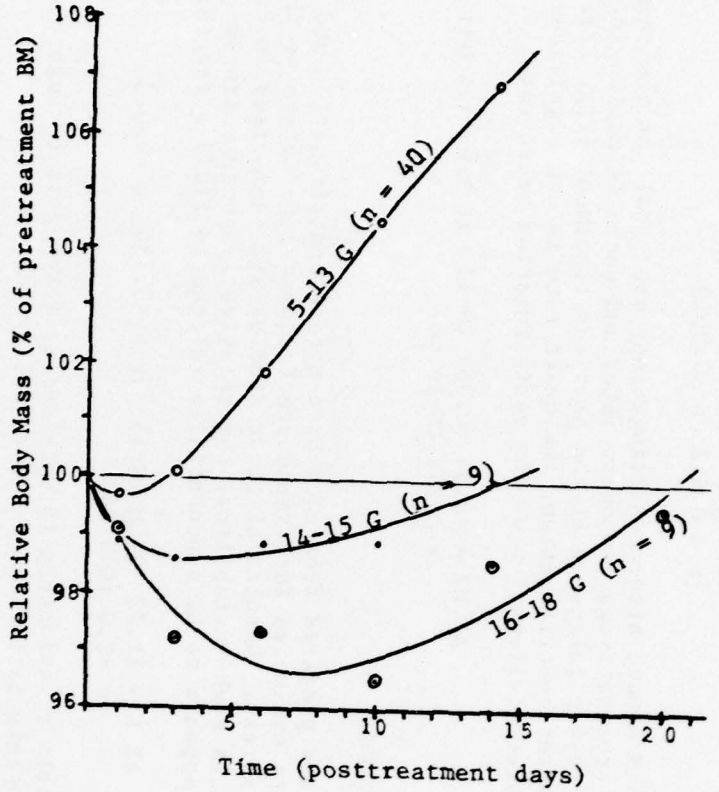


Figure 24. Body mass changes after a 1-minute exposure to HSG. Body masses (as % of pretreatment value) are summarized in three G-field exposure groups.

This response to HSG exposure is restricted to a depressed vegetative function. The animals behaved normally and did not appear to suffer any physical limitations. Feed balance studies were not conducted, so it is not known if this response is one of anorexia or one of an increased maintenance requirement (metabolic rate). Similar changes seen in chronically accelerated animals (28, 29) involve a transient anorexia and an elevated maintenance requirement. (Currently, involvement of endocrine mechanisms is considered likely. The significance of this finding is the implication that even brief exposures to very high acceleration fields (>13 G for chickens) induce a metabolic derangement which may be quite persistent. This phenomenon appears to be unrelated to the myocardial problems, which arise with longer exposure, and it does not appear to involve an overt sickness or organic damage.

Cumulative Effects of Maximum Fields

Since the single 1-minute exposure did not produce any severe mortality or apparent pathology, two groups of birds were exposed to a series of eight 1-minute exposures of 12 G. One group had all treatments in 1 day (4 in the morning and 4 in the afternoon, with 5 minutes between treatments); the other group had the treatment spread over 2 days (2 morning and 2 afternoon exposures each day, with 1 hour between accelerations). The animals were sacrificed and autopsied after the last treatment. Observations from this experimental series are summarized in Table 20.

TABLE 20. CUMULATIVE EFFECTS OF REPEATED 1-MINUTE EXPOSURES TO 12 G

			Lymphocytes		
				After last treatment	
	(n)	Body mass (kg)	Initial (L/100 WBC)	(hrs)	(Δ%)
8 treatments in 1 day	(2)[a]	1.85±0.30	48.5±16.3	4	-54.1±45.8
				24	-0.3±56.9
				48	-26.6±23.5
8 treatments in 2 days	(3)	2.76±0.38	83.3± 5.5	4	-55.5±11.8

	Heart		Abdominal fat pad	Pathology	
Wing band	(g)	(g/100g BM)	(g)	Heart	Other
8 treatments in 1 day:					
48	11.6	7.07	0	NVL	Some hemorrhage, both lungs.
56[a]	15.3	7.03	0	Petechiae[b]	10 ml pericardial fluid; engorged duodenal loop.
95	10.8	5.64	0	NVL	Some hemorrhage, both lungs.
8 treatments in 2 days:					
159	18.7	6.31	16	NVL	Some hemorrhage, both lungs.
177	17.2	7.71	13	NVL	" " " "
984	19.8	7.08	95	NVL	" " " "

[a]One bird died on third acceleration.
[b]Petechial hemorrhages on ventral borders of ventricles.

The eight 1-minute exposures to 12 G were tolerated by most birds. At autopsy, no myocardial changes were evident in the survivors of the treatment--indicating that duration of a single exposure may be more important than field intensity or total exposure time to the induction of such pathology. A rather surprising finding was the uniform appearance of pulmonary hemorrhages--which is not at all common in the other kinds of treatments.

MYOCARDIAL PATHOLOGY

The heart muscle is subject to injury from a variety of causes. Coronary artery disease often results in ischemic necrosis, and a number of infectious diseases will produce primary inflammatory reactions with degeneration of adjacent myocytes. Severe stress from hemorrhagic shock (11,22), forcibly restrained posture (19), and structural alterations in the myocardium (cardiomyopathy) of animals and possibly man (25) will produce myocardial pathology, but the mechanism of heart damage from such stimuli is poorly defined. Similarly, exogenously administered catecholamines produce myocardial injury through a mechanism that is not entirely understood (16). According to one hypothesis, the release of endogenously stored cardiac catecholamines during episodes of stress can result in degeneration of cardiac myocytes. Subsequent fibrosis resulting from repeated injury over a prolonged period may account for some decrease in myocardial functional reserve and may ultimately contribute to the death of a stressed individual.

The remarkable structural similarities between these apparently stress-related (catecholamine-induced) myocardial lesions in man and their counterparts in stressed experimental animals have promoted an interest in an animal-model system suitable to studies of long-term cardiovascular sequelae of high sustained HSG stress. In this program we have examined about 200 birds that had been accelerated according to several schedules. The significant pathological developments are summarized in Appendix E.

Single (Acute) HSG Exposure

Examination of hearts from birds that had received a single exposure to HSG of varying intensity and duration has resulted in the identification of several pathological changes, collectively termed acceleration cardiomyopathy. Such lesions have been described and characterized in both the chicken (30) and minature swine (21), and both species have been proposed as suitable models for investigating potential health effects of HSG on personnel operating high-performance aircraft.

A high percentage of chickens exposed to acute HSG show bright-red, well-delineated subendocardial hemorrhages, which generally are associated with left ventricular papillary muscles. These lesions are characterized by an extravasation of red blood cells between the endocardium and myocardium, which frequently invest bundles of Purkinje fibers (Fig. 25). Adjacent to these areas of subendocardial hemorrhage, severe congestion of myocardial capillaries is also generally apparent. Simple congestion of the myocardial capillaries (SEC) in the absence of subendocardial hemorrhage, another frequent occurrence, may represent early or mild changes in cardiac microcirculation preceding myocardial-subendocardial hemorrhage.

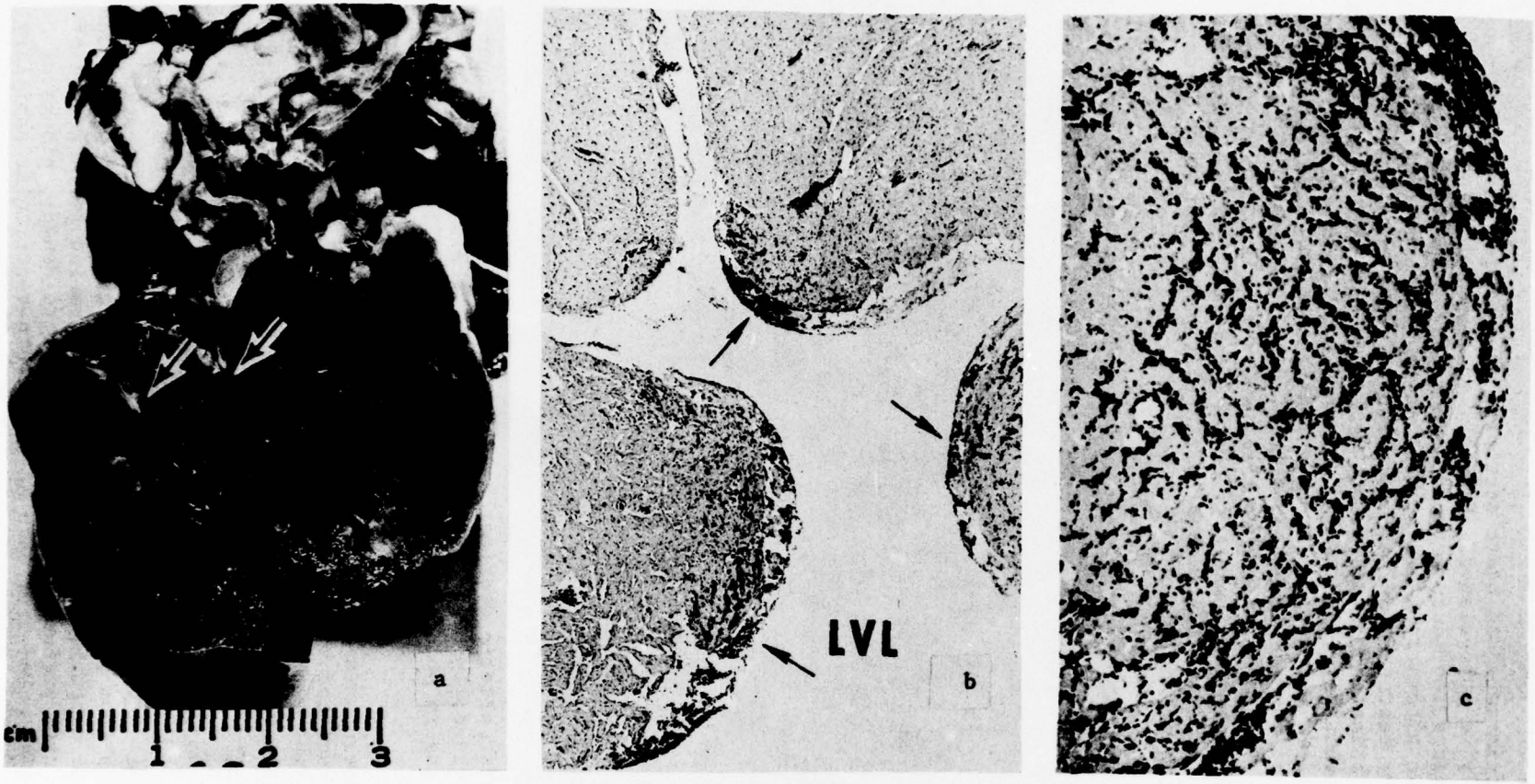


Figure 25. Subendocardial hemorrhage:

a. Gross photograph of the endocardial surface of the left ventricle, showing focal subendocardial hemorrhage (arrows) at the tip of papillary muscles.

b. Low-power micrograph (H&E, x 50) showing subendocardial hemorrhage (arrows)--left ventricular lumen (LVL).

c. Higher power micrograph (H&E, x 125) showing detail of subendocardial hemorrhage surrounding Purkinje fibers, and adjacent to myocardial congestion.

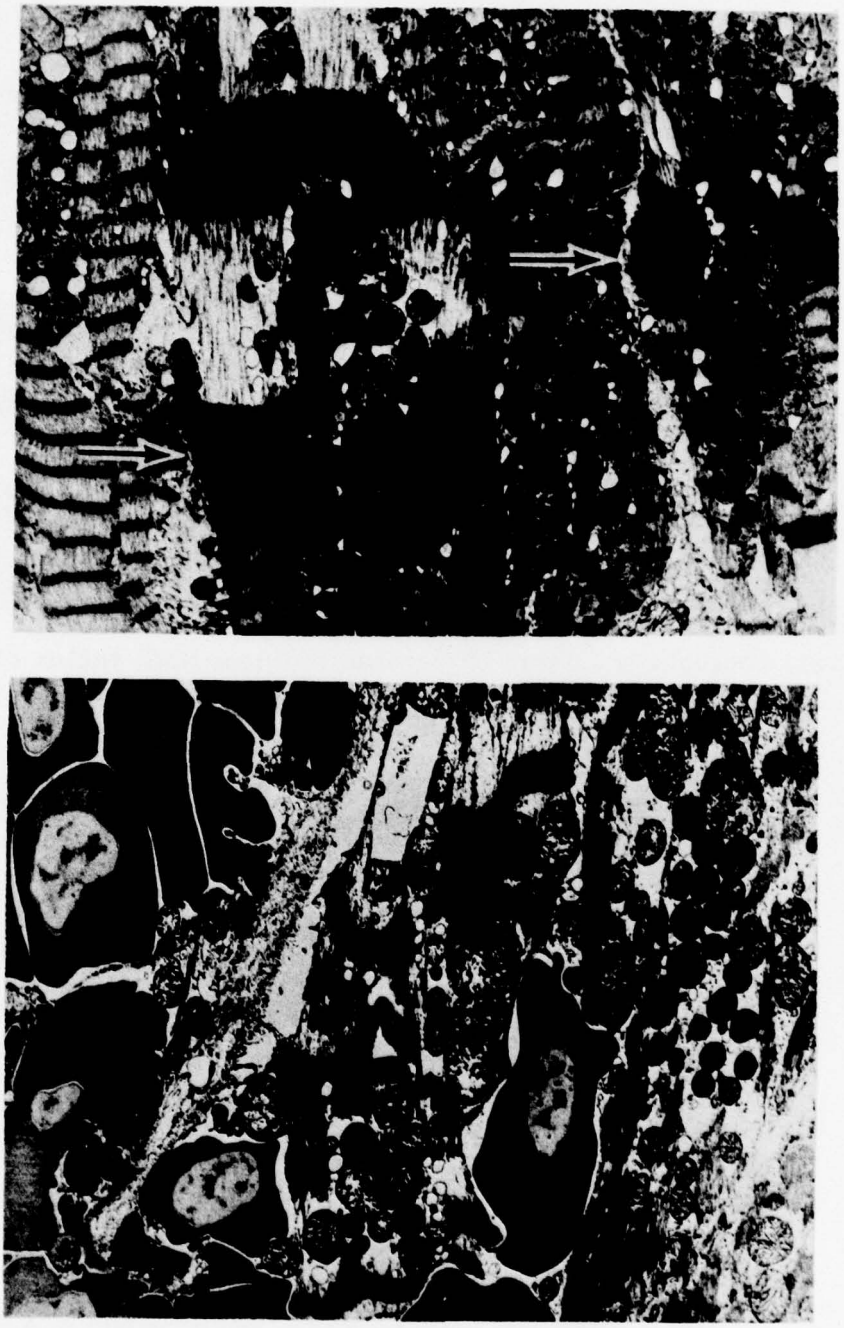
In routine paraffin-embedded heart sections, changes in the cardiac myocytes cannot generally be appreciated. However, in 1-μm toluidine-blue-stained, plastic-embedded sections, structural changes in individual myocytes can be resolved. Such alterations are evident as foci of hypercontracted myocytes characterized by reduction of sarcomere length, with more closely opposed Z bands (Fig. 26). Ultimately, a confluence of Z-band material between several adjacent sarcomeres produces a wide band of dense amorphous material, or contraction band (Fig. 27). Further degenerative changes in cells adjacent to those in which contraction-band formation is taking place consist of severe swelling of organelles (especially mitochondria and sarcoplasmic reticulum), necrosis with infiltration of inflammatory cells (Fig. 28), and dehiscence of intercalated discs (Fig. 29). Intravascular platelet plugs routinely border areas of severe degeneration (Fig. 30).

Figure 26. Characteristic myofiber hypercontraction (arrows) in Epon-embedded, 1-μm section from an area of the myocardium remote to hemorrhages. (Toluidine blue, x 250)

Although the consequences and ultimate resolution of these degenerative myocardial lesions associated with HSG are currently unknown, further studies are in progress to define pathological changes occurring in the hearts of chickens undergoing chronic HSG.

Recurrent (Chronic) HSG Exposure

In the final phase of this study, the cumulative effects of repeated HSG were examined in hearts from 9 birds, segregated into 3 groups of 3 birds each which were subjected to differing intensities of acceleration for a period of 6 months. Following in situ perfusion with cold 2% gluteraldehyde, blocks of myocardial tissue were selected from each of the 3 left-ventricular papillary



a

b

Figure 27. Abnormal distribution (translocation) of mitochondria. (x 5,000)
a--Many mitochondria are swollen and contain large flocculent densities. Sarcomere I bands have disappeared, indicating hypercontraction. Sarcomere Z-band material has become confluent in several areas, forming contraction bands (arrows).
b--Similar but more severe changes in the heart from an area of intramyocardial hemorrhage.

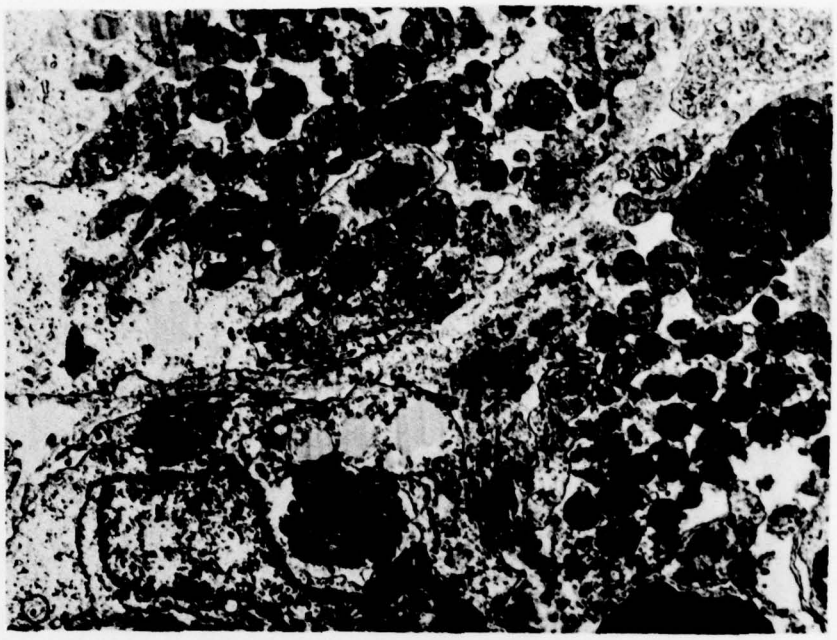


Figure 28. Necrosis and lysis of cardiac myocytes with influx of inflammatory cells. (x 6,000)

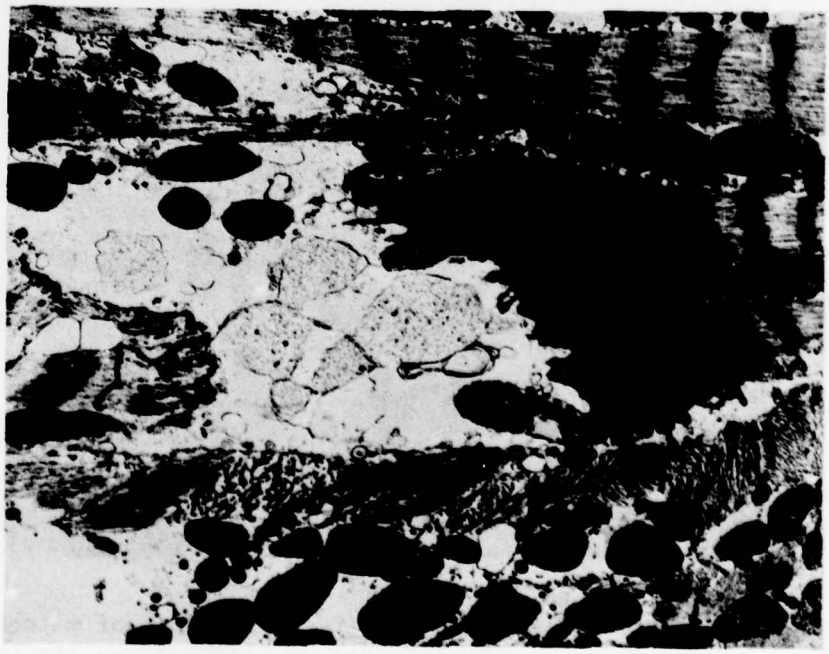


Figure 29. Dehiscence of an intercalated disc in area of myocardial injury. (x 8,000)

Figure 30. Epon-embedded, 1-μm section from an area of myocardial hemorrhage. Note the hypercontracted state of the myofibers, the decreased staining affinity of the cells, and the granular nature of the sarcoplasm (swollen mitochondria). A platelet plug is present in a small vessel (arrow). (Toluidine blue, x 250)

muscles and prepared for electron microscopy. From these birds, 129 blocks of myocardial tissue were examined in 1-μm toluidine-blue-stained sections by light microscopy.

In 13 of the 129 blocks examined, myocardial capillaries in some regions contained numerous red blood cells and appeared to be inadequately perfused. Adjacent sections of tissue showed empty and widely dilated capillaries consistent with adequate perfusion. Although unperfused areas of the heart had a distinct morphologic appearance, the underlying changes associated with acceleration cardiomyopathy were not obscured. However, because of the apparent lack of perfusion, these 13 blocks were excluded from analysis.

To represent the spectrum of pathological changes in the hearts, 12 blocks were selected on the basis of their appearance under light microscopy and subsequently were examined ultrastructurally.

Pathological changes observed in the well-perfused blocks had a patchy distribution within the papillary muscle. A marked difference in the relative severity of change, as indicated by the distribution and frequency of lesions, characterized the three treatment groups. Birds receiving 6 G for 4 minutes, 8 times daily, showed the most severe myocardial alteration; while those receiving 8 G for 1 minute, 4 times daily, showed the least:

Wing band No.	Treatment (6-month period)	Relative pathologic severity
91 107 137	6 G, 4 min, 8 times daily	3
69 227 228	4 G, 2-5 min, 4 times daily	2
1 140 190	5-8 G, 1 min, 4 times daily	1

Three pathological changes were encountered most often in birds subjected to chronic HSG. Light microscopically, irregular areas of the myocardium demonstrated less intense staining properties. In these areas, myofibrillar detail (cross-striations and longitudinal fibrils) was obscured. Surrounding myocytes often showed normal cytoarchitecture. The altered areas often radiated into the myocardium from the adventitia of small vessels, but they also could be observed in regions not associated with the vasculature. These damaged areas were interpreted as foci of fibrous tissue proliferation and most likely represent areas of previous myocardial degeneration with fibrous resolution (Figs. 31, 32). As a second distinct change, cardiac myocytes immediately surrounding the fibrous areas showed a marked increase in the frequency of intercalated discs (Fig. 33). This alteration may represent an adaptive response by the injured myocardium towards structural reinforcement of intercell association--which is important to the interaction and normal function of cardiac myocytes. Thirdly, focal areas of hypercontraction were encountered frequently, some showing contraction-band formation with the dissolution of myofibrillar architecture (Fig. 27a). These changes often affected individual myocytes, with obvious redistribution of mitochondria, and caused the surrounding, more normal myocytes to take on a wavy, distorted appearance. Subendocardial hemorrhage or evidence of previous (resolved) hemorrhages could not be detected in birds repeatedly exposed to HSG over several months.

The subtle changes noted in the birds that were centrifuged repeatedly would be difficult to detect in routinely prepared, paraffin-embedded, H&E-stained material. Our findings suggest, however, that recurrent injury of sufficient magnitude eventually will produce degenerative changes that may be detectable with routine methods and may result in some degree of cardiovascular impairment.

Although this study of HSG-induced cardiovascular injury involved only a few birds within a restricted treatment regimen, the pathologic lesions demonstrated are identical to those occurring in acceleration-stressed pigs (30) and in stress cardiomyopathy in humans (25). The differences in the frequency or severity of lesions in the hearts of three groups of accelerated birds is suggestive of a dose-response relationship.

Figure 31. Myocardial fibrosis area deep in the heart muscle: Epon-embedded, 1-μm section. (Toluidine blue, x 250)

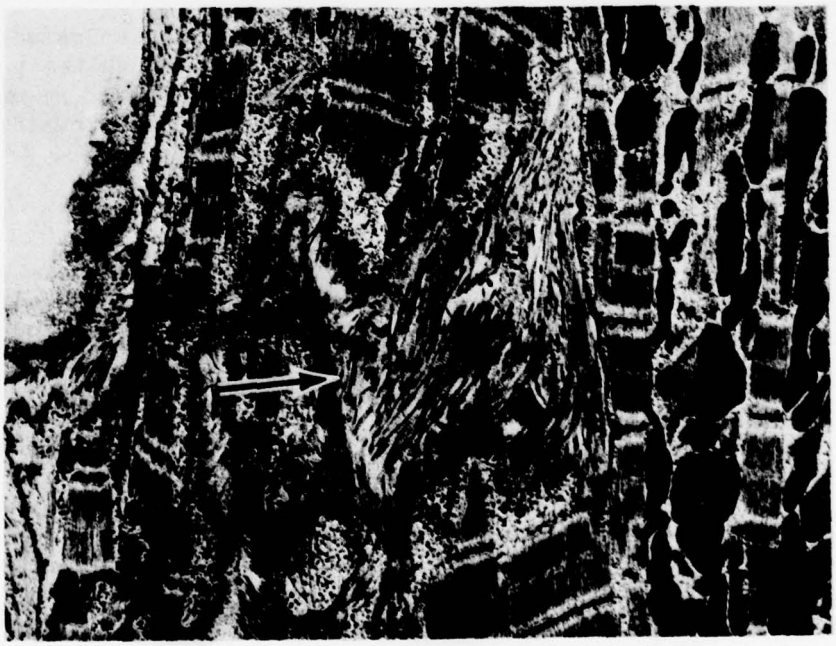


Figure 32. Myofiber disruption by irregular proliferation of mature collagen: electron micrograph of area similar to Fig. 31. (x 5,000)

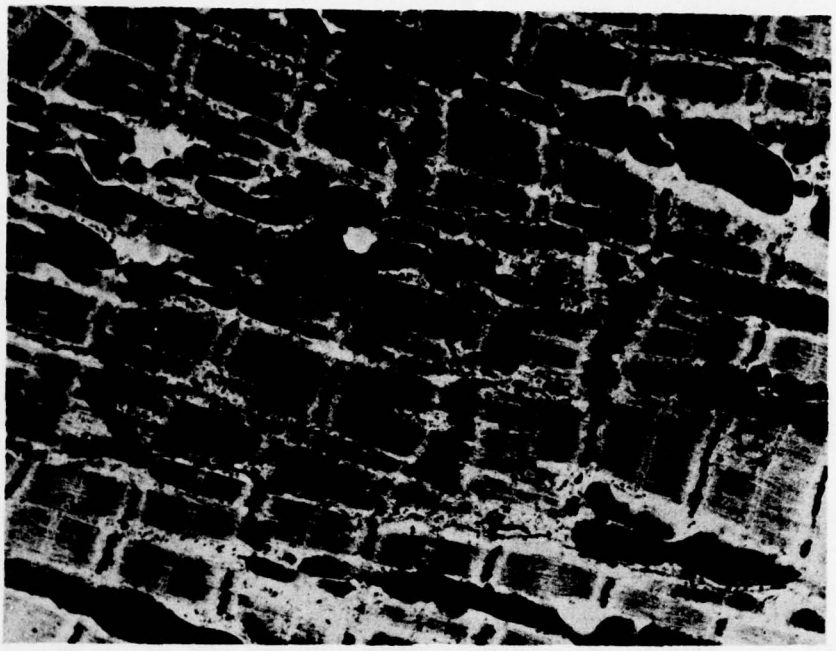


Figure 33. Myocardium area showing increased number of intercalated discs. (Electron micrograph, x 7,500)

We believe this investigation establishes the chicken as a suitable model for studying acceleration-induced cardiomyopathy, although the pathogenesis of changes and their magnitude under various conditions should be understood in greater detail before attempting to extrapolate such data toward predicting human responses to HSG.

OUTLOOK

The results of this program indicate that the domestic fowl provides a suitable human surrogate for acceleration studies. Its size and availability makes it a convenient subject. It also has a range of acceleration tolerance that is related systematically to biological factors (such as age, sex, body size, and species body size) as well as to acceleration-field strength. Considerable individual variability exists, in which circulatory phenomena appear to be determining factors. The bipedal posture of the fowl provides it with circulatory characteristics (e.g., a visceral vasomotor apparatus) that are systematically different from those of quadrupeds, and resemble more closely the human pattern.

When the fowl is exposed to acceleration schedules of sufficient intensity, duration, and frequency, various pathological changes occur. Some of these are grossly apparent lesions in the subendocardium, which appear to be transient; and others are found only submicroscopically. These acceleration cardiomyopathies are essentially similar to pathological changes found in centrifuged mammals. The relationship between the gross and submicropathologies has not been established, but continuing examination of tissues on hand should help clarify the sequence of pathogenesis.

There also is evidence of a separate pathophysiology that results from even brief exposure to very intense fields (e.g., 1 minute in fields >15 G). This effect is characterized by a repression of growth--which is selective, since the animals otherwise had the appearance of normalcy. Such changes are seen during (but not after) chronic acceleration (28,29) and have been shown to result from a transient anorexia. This pattern is very similar to sequelae from experimental injury (i.e., electrocautery) to the lateral hypothalamus (LH). Intense fields may affect the hypothalamic region mechanically and induce changes in the food-intake regulating centers, which are known to be located therein. The mechanisms for this phenomenon relate to the brain load (negative buoyancy of the brain), which is proportional to the difference in specific gravity between brain and cerebrospinal fluid and the ambient acceleration-field intensity. So, in a field of 15 G, the brain load (a force which must be borne by some brain tissue) is 15-fold greater than at Earth gravity. Such a force might lead (at least temporarily) to changes resembling LH lesioning. It may be important to investigate this syndrome further, as a potential acceleration injury that would be independent of circulatory factors (i.e., unaffected by protective devices that have a circulatory basis).

This research program has suggested a variety of follow-on experiments which may warrant further attention; for example:

Factors Affecting Acceleration Tolerance: One result of the present study is an evaluation of the acceleration tolerance of the fowl. It might be productive to extend this investigation into the influence of pharmacological and similar agents upon acceleration tolerance. Of particular interest in this regard would be social toxicants (e.g., aspirin, barbiturates, caffein, cannabinol, ethanol, nicotine), industrial toxicants (e.g., carbon monoxide and fuel hydrocarbons), and medications (e.g., androgens, antihistamines, estrogens, insulin, propranolol, reserpine). Some of these materials are encountered, commonly or rarely, by flight personnel, and others are agents that have been considered by many to affect acceleration tolerance.

The effect on acceleration tolerance could be studied at three levels: (1) The incidence of susceptibility--the mortality observed in the screening procedure; (2) the period of maintenance of a normal heart rate in a 6-G field--the time until a bradycardia; and (3) the response to a short exposure to a very intense field (1 minute at 16-18 G), such as degree of lymphopenia or subsequent maintenance of body mass.

Neck Length and Body Orientation as Determinants of Tolerance: The present restraint harness provides adequate support to the centrifuging animal, but it does allow for some variation in neck length--which may contribute to the interindividual variability of acceleration tolerance. We have considered the possibility of fixing neck length by adding a cylinder of Orthoplast (a low-density thermoplastic material used for splints) to the present harness. Experiments would then be feasible to determine the influence on acceleration tolerance of such factors as (a) neck length--the "vertical" eye-to-heart distance; (b) body orientation--the angle of the principal body axis to the acceleration field; and (c) leg extension--as a contributor to vascular column length.

Functional Impairment of Myocardial Pathology: As the production of myocardial lesions becomes more predictable, it should be possible to measure the limitation, if any, they have upon physical performance. The exercise capacity (running to exhaustion) has been determined for the domestic fowl (23), and procedures and equipment (treadmills) are on hand to carry out exercise studies on animals with lesioned hearts. These facilities could be used also to prepare physically trained birds, to test the influence of physical fitness upon susceptibility to cardiomyopathies. With chickens, daily exercise (two half-hour periods) produces a significant physical conditioning--whereas running to exhaustion at weekly or longer intervals does not appear to improve performance.

At the organ level, determining the influence of heart lesions upon cardiac function would be quite feasible. Of particular interest would be the conductile properties, which could be investigated by methods as described by Professor Goldberg in Appendix B. This may be particularly important, since functional impairment may not be strictly proportional to the degree of lesioning. The degree of functional limitation could be compared with histologically evaluated severity of lesioning--also, this relationship could be examined at various times in the development and resolution of the lesions.

ACKNOWLEDGMENT

We gratefully acknowledge the special efforts of Ms. Ina Green in the preparation of this manuscript.

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APPENDIXES A - D

APPENDIX A. ACCELERATION TOLERANCE IN MALE CHICKENS

Three screening fields (4, 6 and 8 G) were used so that the results would overlap older (and more susceptible) birds as well as lighter breeds (Leghorns) which are more tolerant. Score describes survivors' physical appearance after centrifugation [see page 14]. Body mass (kg) and postacceleration score are cited as group means ± standard deviation.

				Initial group characteristics		Tolerance				
						Survivors			Nonsurvivors	
Intensity	Strain	Age (days)	Accel. treat. (min)	(n)	Body mass	(n)	Body mass	Score	(n)	Body mass
LOW (4 G)	RIR-3	76	<4	(30)	1.28±0.18	(30)	1.28±0.18	1.77±0.62	(0)	
			4-6	(30)	1.28±0.15	(30)	1.28±0.18	1.47±0.67	(0)	
			6-8	(30)	1.29±0.18	(29)	1.27±0.14	1.55±0.56	(1)	1.88
			8-10	(29)	1.27±0.16	(28)	1.27±0.16	1.24±0.43	(1)	1.22
			10-12	(28)	1.47±0.15	(27)	1.47±0.15	1.29±0.45	(1)	1.48
			12-20	(27)	1.45±0.17	(25)	1.46±0.17	1.33±0.47	(2)	1.31±0.01
	RIR-3	127	<12	(30)	2.34±0.24	(29)	2.34±0.24	1.10±0.30	(1)	2.53
			12-20	(29)	2.33±0.23	(28)	2.32±0.23	1.25±0.43	(1)	2.67
INTERMED. (6 G)	RIR-3	76	<4	(60)	1.11±0.12	(55)	1.10±0.12	1.96±0.19	(5)	1.20±0.09
			4-8	(55)	1.10±0.12	(41)	1.09±0.13	2.12±0.71	(14)	1.12±0.11
	RIR-2	83	<4	(183)	1.24±0.23	(167)	1.23±0.24	Not scored	(16)	1.34±0.19
			4-8	(167)	1.23±0.24	(131)	1.21±0.24	" "	(36)	1.32±0.18
	RIR-3	127	<4	(30)	2.22±0.18	(20)	2.16±0.15	2.10±0.99	(10)	2.32±0.18
			4-8	(20)	2.21±0.15	(12)	2.14±0.14	2.33±0.75	(8)	2.31±0.99
	RIR-1	289	<4	(53)	3.14±0.28	(41)	3.08±0.28	Not scored	(12)	3.36±0.18
			4-8	(41)	3.08±0.28	(23)	3.03±0.26	" "	(18)	3.13±0.29
HIGH (8 G)	RIR-3	76	<2	(60)	1.13±0.13	(58)	1.12±0.13	1.98±0.99	(2)	1.24±0.12
			2-3	(28)	1.24±0.14	(19)	1.23±0.15	1.84±0.87	(9)	1.25±0.11
			3-4	(30)	1.22±0.12	(16)	1.24±0.12	2.19±0.81	(14)	1.19±0.12
	RIR-3	127	<2	(30)	2.37±0.23	(23)	2.30±0.20	2.57±1.21	(7)	2.61±0.16
			2-3	(11)	2.28±0.17	(9)	2.23±0.14	3.56±1.07	(2)	2.52±0.47
			3-4	(12)	2.35±0.22	(3)	2.16±0.85	3.00±1.63	(9)	2.41±0.22

INTERMED. (6 G)	Leghorn	96	<4	(36)	1.12±0.14	(36)	1.12±0.14	1.92±0.50	(0)	
			4-8	(36)	1.12±0.14	(35)	1.13±0.14	1.80±0.41	(1)	1.08
			8-12	(35)	1.17±0.14	(35)	1.17±0.14	1.86±0.55	(0)	
	Leghorn	165	<4	(65)	2.04±0.24	(64)	2.05±0.24	Not scored	(1)	1.74
			4-8	(64)	2.05±0.25	(61)	2.06±0.24	" "	(3)	1.85±0.18
			8-12	(61)	2.06±0.24	(58)	2.05±0.24	" "	(3)	2.20±0.12
	Leghorn	223	<4	(6)	1.80±0.31	(6)	1.80±0.31	2.00±0.63	(0)	
			4-8	(12)	1.84±0.23	(11)	1.86±0.24	2.18±0.60	(1)	1.68
			8-12	(12)	1.66±0.15	(12)	1.66±0.15	2.17±0.39	(0)	
	Leghorn	465	<4	(36)	2.22±0.30	(36)	2.22±0.30	Not scored	(0)	
			4-8	(36)	2.22±0.30	(33)	2.19±0.29	" "	(3)	2.36±0.13
			8-12	(33)	2.19±0.29	(30)	2.20±0.27	" "	(3)	2.01±0.36
HIGH (8 G)	Leghorn	146	AM 4	(17)	1.62±0.23	(17)	1.62±0.23	1.71±0.69	(0)	
			PM 4	(17)	1.64±0.13	(16)	1.64±0.13	2.00±0.73	(1)	1.69
	Leghorn	178	2-4	(53)	2.06±0.25	(41)	2.02±0.22	Not scored	(12)	2.16±0.27
	Leghorn	223	<2	(12)	1.79±0.19	(12)	1.79±0.19	1.75±0.45	(0)	
			2-4	(24)	1.76±0.19	(18)	1.75±0.17	2.11±0.47	(6)	1.81±0.23

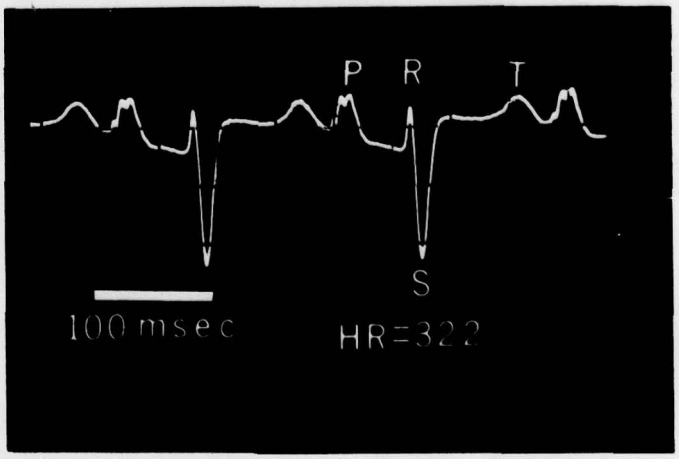
APPENDIX B: ELECTROCARDIOGRAM OF THE CHICKEN AND ACTIVATION OF THE CHICKEN HEART

J. M. Goldberg

The electrocardiogram of the chicken has been recorded by a number of investigators (Goldberg and Hill, 1977; Lewis, 1915; Kisch, 1951; and Sturkie, 1949). In those experiments the leads were connected to the base of each wing and to the left leg, corresponding to the electrode sites used in man and dog. We found these sites to be unsatisfactory for recording consistent ECGs with birds during centrifugation. We have employed a single-lead system with one electrode placed through the skin on the dorsal surface of the neck and the other dorsally at the base of the tail.

Morphology of the ECG

The ECG of the chicken consists of a waveform corresponding to the activation of the atria, a complex associated with ventricular activation, and a wave representing repolarization of the ventricles. The morphology of these waveforms is different from that observed with man and dog. The amplitudes of the waves, representing atrial and ventricular depolarization and ventricular repolarization, are smaller for the chicken because its heart mass is less than that of man or dog. The P wave has variable morphological characteristics, but generally consists of an upright segment which may be bipeaked, tripeaked, biphasic, or notched. The ventricular activation complex is unique, as compared to the mammalian pattern. The dominant component is a large downward deflection which may be preceded by a smaller upright wave of variable amplitude. Ventricular repolarization represented by an upright segment in the following figure illustrates the ECG as recorded with the back lead system.



The nomenclature designating the waves corresponding to atrial depolarization and ventricular repolarization is the same as that used in mammalian electrocardiography: P designates atrial depolarization, and T, ventricular repolarization. However, two conventions have been used to designate the components of ventricular activation. In one, the initial positive wave is designated R, and the following negative wave is the S wave. This system uses the

polarity of the mammalian QRS complex for designating the corresponding waves in the chicken ECG. The second convention terms the first component the Q wave and the second the R, irrespective of the polarity of the waveforms. With this convention the initial small upright wave would be termed the Q wave; and the major downward deflection following, the R segment.

Anatomy of the Avian Heart

The avian heart is four chambered, but unlike the mammalian heart, there are three sets of valves at the entrance of the right atrium and three vena cavae. Two of these sinoatrial valves (SA valves), those at the right and left anterior cavae, exhibit pacemaker activity. Thus, two areas in the avian heart consistently exhibit diastolic depolarization, as compared to one, the SA node, in the mammalian heart. The structure that corresponds to the atrioventricular (AV) node in the mammalian heart is situated in the upper ventricular septum rather than in the interatrial septum, as in mammalian species.

The avian heart has a more extensive Purkinje network than do mammalian hearts. In addition to the Purkinje network found in the septum and endocardial surface of the mammalian heart, avian hearts have Purkinje fibers associated with the ventricular coronary vessels, the muscular right atrioventricular valve, and the atria musculature. This extensive network of Purkinje fibers is important in determining the pattern of atrial and ventricular activation.

Activation of the Heart and the Electrocardiogram

Only one study has mapped atrial activation in the avian heart, and correlated its sequence with the morphology of the P wave (Goldberg and Hill, 1977). It was observed that the initial site of activation in the chicken heart is localized nearest the right SA valve. Moore (1965) has shown that the regions of both the right and left SA valves have cells exhibiting the characteristic pacemaker prepotential.

In the studies of Goldberg and Hill (1977), positioning the exploring electrode near the left SA valve was difficult, so this region was not investigated as extensively as was the right SA valve. The epicardial mapping of the chicken heart demonstrated that the spread of activation through the atria was not radial. Instead, islands of atrial tissue became activated, and their wavefronts then coalesced. Most of the right atrium was activated before left atrial excitation was initiated. The nonradial spread of activation in the atria may be related to the existence of muscle bundles interconnecting regions of the right atrium and to the pervasive atrial Purkinje network (Davis, 1930). However, preferential internodal pathways comparable to those found in dog and man have not been identified in avian hearts.

The studies of Goldberg and Hill (1977) found that the P waves observed with activation of the chicken heart can be divided into 8 types based upon waveform morphology. The most frequent waveform was bipeaked, with peaks of equal amplitude, which reflects the sequential activation of the two atria. Because of the time required for mapping atrial activation, it was impossible to determine the changes in the remainder of the pattern associated with each P-wave morphology. In many experiments the P wave was quite labile; and while

the initial site of activation could not be determined for each type, the changes in morphology probably reflect pacemaker shifts within the atria leading to a change in the pattern of atrial excitation.

Little is known about atrioventricular conduction in the avian heart. There is an isoelectric period from the end of the P wave to the beginning of the R or S wave--a delay between the termination of atrial activation and the onset of ventricular activation. Moore (1965) observed that in the turkey heart, increasing the frequency of atrial pacing prolonged atrioventricular conduction time--a response similar to that observed in mammalian preparations. Therefore, some avian structure functionally behaves as does the AV node in mammalian hearts.

Ventricular activation in the bird heart has been mapped by Lewis (1915), Kisch (1951), and Moore (1965). The pattern of activation differs from that found in man and dog in that the epicardium is activated early and very rapidly, with ventricular activation directed mostly from the apex to the base. While the right ventricle is activated slightly ahead of the left, the activation of both ventricles is almost simultaneous. The early activation of the epicardium and the synchrony of ventricular activation can be attributed to the very extensive and rapidly conducting Purkinje fiber system.

Neural Control of the Avian Heart

Few of the techniques used to study neural control of mammalian hearts have been applied to birds. Using pharmacological blocking agents, Sturkie (1970) demonstrated that the heart rate of the chicken at rest represents a tuning of both sympathetic and parasympathetic activity. Both divisions of the autonomic nervous system are active, and the heart rate represents the summation of these two opposing chronotropic effects. This differs from mammalian control systems in which the parasympathetic effect dominates under resting conditions. However, the details of potential sympathetic and parasympathetic control of heart rate and pacemaker localization have not been defined for avian hearts.

Even less is known about any neural control of atrioventricular conduction and ventricular conduction in bird hearts. Moore (1965) observed bradycardia but no prolongation of atrioventricular conduction when he stimulated the right and left vagi in the turkey. However, with the bradycardia the margin of safety for conduction between the atria and ventricles may have been sufficient to preclude any delay in conduction. In centrifuging chickens, prolongation of the P-S interval of the ECG has been observed along with the bradycardia, so atrioventricular conduction is altered by some means. There is no description of any neural influence on ventricular conduction.

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APPENDIX C: INDIVIDUAL ACCELERATION TOLERANCE, +6 G_z

Animals were centrifuged at 6 G until a marked bradycardia appeared -- taken as the tolerance limit. Of the 61 animals examined, 43 survived.

Wing band No.	Age (days)	Body mass (kg)	Tolerance (min)	Heart rate		Respiratory freq.		Rel. heart size (g/kg)
				Precentrifuge Initial	Immed. change Δ%	Precentrifuge Initial	Immed. change Δ%	
SURVIVING TREATMENT								
19	335	3.11	3.0	287	-2	--	---	4.7
22	335	2.49	1.5	352	-8	--	---	5.6
35	337	3.32	10.5	344	-24	--	---	5.0
42	335	3.19	5.5	298	-2	--	---	6.2
59	134	2.16	17.0	395	-1	27	+70	---
73	335	2.88	11.5	333	-31	--	---	6.1
75	155	1.77	70.0	337	0	22	+68	---
93	335	2.78	4.5	342	-44	--	---	4.8
119	134	2.59	8.5	349	0	33	+185	---
132	335	3.30	6.5	352	-4	--	---	4.9
200	154	1.84	18.5	372	-11	24	-8	---
251	154	2.75	11.5	281	+5	25	+68	---
1509	132	2.55	2.0	321	+17	--	---	5.9
1563	111	2.30	5.0	338	+15	--	---	5.8
1576	107	1.83	6.0	336	+10	--	---	5.4
1582	111	2.00	7.5	389	-8	--	---	4.5
1590	132	2.48	7.0	357	-12	--	---	5.5
1605	111	1.93	4.0	324	-2	--	---	5.3
1617	107	2.05	16.0	372	+7	--	---	5.8
1618	132	2.72	1.0	360	-38	--	---	4.6
1632	107	1.98	11.5	352	-1	--	---	4.3
1645	113	1.90	19.0	396	+8	--	---	5.0
1646	111	2.07	20.5	357	+13	--	---	4.6
1661	112	1.77	22.0	362	-1	--	---	5.4
1695	112	2.13	6.0	307	+14	--	---	5.2
1708	132	2.53	9.5	294	-7	--	---	4.8
1728	118	2.21	13.0	412	+7	--	---	4.4

2628	258	3.25	4.0	286	-27	25	-50	---
2723	615	3.30	2.5	330	-17	—	---	5.6
2734	257	3.60	15.0	282	-22	25	-60	---
2775	249	3.30	14.0	320	-19	27	+133	---
2837	249	2.93	14.0	280	-21	37	-19	---
2845	249	2.80	40.0	290	+19	35	+100	---
2861	245	3.60	9.0	330	-24	--	---	---
2866	251	2.93	10.0	260	+23	18	+105	---
2868	244	3.50	30.0	357	-6	30	+67	---
2884	272	3.32	13.0	254	+18	45	-33	---
2896	264	2.76	5.0	220	-9	30	+67	---
2897	251	3.31	15.0	340	0	40	+18	---
2956	265	3.64	5.0	300	-20	30	+67	---
2958	257	3.35	1.0	225	+11	15	-60	---
2969	257	3.66	15.0	342	-9	20	+25	---
2979	272	2.92	15.0	270	+11	30	---	---
NOT SURVIVING TREATMENT:								
4	337	3.98	5.0	332	+5	--	---	4.7
54	335	3.41	10.0	349	-17	--	---	5.8
84	335	3.20	10.0	351	+6	—	---	6.0
1583	112	1.91	16.0	382	+5	—	---	4.7
1593	109	2.20	6.5	322	-2	--	---	5.3
1607	107	2.30	14.0	329	+3	--	---	5.4
1625	132	2.99	18.0	292	+5	--	---	6.3
1642	109	2.02	11.5	358	-3	--	---	5.8
2727	272	3.85	13.0	300	-50	45	-67	---
2752	251	3.80	5.0	295	+4	55	-46	---
2771	272	2.90	4.0	360	-36	31	-100	---
2887	272	3.24	4.0	330	-42	38	-15	---
2889	615	3.57	3.5	225	-24	--	---	5.3
2899	272	3.90	4.0	230	-13	—	---	---
2900	615	3.38	5.0	352	-27	—	---	5.3
2901	265	3.52	5.0	280	+39	20	-50	---
2915	251	3.41	4.0	262	+19	62	-60	---
2999	272	3.78	10.0	330	-46	30	+200	---

APPENDIX D. PHYSIOLOGICAL RESPONSES TO A 1-MINUTE EXPOSURE TO $+G_z$

Field (G)	Wing band No.	Body mass (kg)	Days of age	Lymphocytes: Initial (%)	Lymphocytes: 4 hrs after (Δ %)	Initial heart (bpm)	Change in heart rate (Δ%): Centrifugation 0-20 (sec)	Centrifugation 0-60 (sec)	Postcentrifugation 0-20 (sec)	Postcentrifugation 0-60 (sec)	Postcentrifugation 60-120 (sec)
	19	2.56	224	84	-33	242±6	+14	+5	-7	-9	-20
5 <	35	2.84	224	76	-16	300±6	+6	-14	-14	-11	-5
	132	2.74	224	74	-35	300±5	+25	+14	+5	+5	0
	87	2.98	224	79	---	310±7	-20	-36	---	---	---
6[a] <	106	1.99	224	61	---	300±7	+31	+19	---	---	---
	202	2.75	224	76	---	316±5	+3	-6	---	---	---
	54	2.96	224	70	-16	286±18	+12	0	-12	-13	-18
7 <	73	2.48	224	78	-28	310±11	+8	-3	-6	-6	-13
	93	2.11	224	54	-28	322±9	+1	-9	-9	-5	-7
	70	2.09	101	66	+5	357±12	+5	+1	-10	-6	-8
	177	1.82	101	62	-13	338±12	+7	+1	+4	+2	+3
	184	1.49	101	73	+3	357±10	+6	-11	-29	-3	+8
8 <	1506	2.61	203	46	-54	309±31	0	-6	-5	+2	+4
	1521	2.53	203	78	-47	247±10	+29	+1	+19	+18	+2
	1522	2.55	203	63	-21	269±70	+23	+6	+5	+1	-7
	1564	2.63	203	72	-64	291±6	-3	-37	-44	-15	+3
	1608	2.67	203	69	-33	302±16	-5	-37	-22	+3	+17
	1707	2.62	203	76	-11	320±48	+18	+3	-9	-2	-3
	19	1.70	102	62	0	322±12	+12	+10	+21	+15	+12
9 <	22	1.41	102	67	-9	330±20	+17	-4	-8	-3	+5
	108	1.83	102	75	-38	336±15	+4	-7	-15	-10	+2
	42	1.87	102	65	-38	323±20	-1	-2	+1	+6	+7
	132	1.46	102	71	-29	379±13	+3	+3	+6	+8	+4
	158	1.99	102	85	-40	313±2	+7	-1	+4	+7	---
	1514	2.65	141	69	-64	285±10	+11	-5	---	+26	+16
	1518	3.65	204	67	-45	222±7	0	-32	-52	-15	+22
10 <	1537	3.33	204	66	-42	251±9	+9	-24	-40	-8	+5
	1551	3.04	204	62	+16	299±2	0	-38	-42	-14	-6
	1581	3.09	204	54	-15	266±5	+12	-17	-38	-15	+3
	1601	2.63	204	83	-37	290±16	-4	-11	-16	-5	+1
	1707	2.37	141	82	-35	340±8	+10	-1	-4	-1	-1
	1715	3.03	204	32	0	285±24	+1	-9	-31	-21	-10
	1716	2.75	141	79	-68	338±23	+7	-29	-47	+24	+1

		48	1.13	118	65	-47	320±5	-25	-28	-19	-15	-6
11	<	56	1.37	118	68	-12	347±9	+14	+10	+7	+1	-2
		95	2.05	118	70	-36	327±4	+7	-12	+10	+15	+9
		19	2.27	152	63	-44	258±10	+23	-26	-16	-18	-6
		132	2.18	152	68	-43	318±9	+15	-14	-15	-3	-1
		159	2.43	118	71	-14	318±2	-1	-31	-47	-27	-21
12	<	177	2.12	152	75	-43	295±8	+4	-21	-7	-3	-16
		180	1.57	118	78	-29	331±17	+13	+14	0	+4	-6
		984	1.89	118	77	-23	316±15	+21	0	+25	+19	+9
		35	2.43	137	78	-32	359±14	-8	-39	-21	-16	-1
13	<	54	2.33	137	49	-8	342±6	0	-1	-6	+2	-4
		93	1.86	137	70	-47	347±10	+2	-5	-20	-7	+11
		87	2.54	152	62	-20	312±21	-34	-43	-46	-39	-24
		73	2.40	152	78	-46	300±23	-30	-44	-55	-41	-15
14	<	106	2.42	152	58	-22	326±18	+15	+18	-61	-36	-8
		1506	2.40	154	67	-43	303±9	-34	-48	-46	-36	+11
		1521	2.34	154	49	-14	322±5	-15	-44	-44	-11	+9
		1715	2.88	154	80	-29	312±14	+11	-4	-4	+8	+9
		22	2.06	194	74	-67	330±12	-17	-42	-58	-36	-32
15	<	70	2.25	194	64	-10	225±0	+13	-27	-33	0	+4
		84	2.80	194	81	-58	282±4	+30	-10	-34	-5	-2
		42	2.95	195	59	-76	252±6	+3	-4	-46	+4	+17
16	<	108	2.70	195	44	-25	326±12	+6	-27	-18	-15	-6
		158	2.55	195	84	-45	273±22	-18	-35	-33	-22	-13
		22	2.44	294	85	-64	317±4	-12	-34	---	-21	-9
17	<	84	3.27	294	81	-59	314±5	+7	-28	-52	-31	-1
		158	3.30	294	88	-59	238±8	-38	-40	-36	-24	-11
		1522	2.36	140	84	-39	327±9	-8	-34	-49	-29	+7
18	<	1672	2.37	140	80	-45	321±4	-2	-36	-41	-28	+5
		1694	2.04	140	79	-32	330±1	-8	-33	-31	-7	+

[a] Animals were centrifuged 1.5 minutes.

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