Spaceflight and Bone Turnover: Correlation with a New Rat Model of Weightlessness

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The near-weightless environment of orbital flight has produced certain biomedical effects in humans including abnormalities in mineral metabolism. Alterations in calcium homeostasis were suggested by early manned spaceflights (Biryukov and Krasnykh 1970; Lutwak et al. 1969; Mack and LaChance 1967; Rambaut et al. 1975), but the most definitive data to date became available following the long-duration flights of the Skylab series (Leach and Rambaut 1977; Vogel et al. 1977; Whedon et al. 1977). The Skylab mineral studies indicated that during flight, urinary calcium increased more rapidly and stabilized within 30 days at a level about twice the preflight value (Rambaut et al. 1979 and Fig. 1). Fecal calcium decreased initially, then continued to rise throughout the flight with no indication of abating. Calcium balance returned to normal in the postflight period.

These data were compatible with bone mineral loss. The relative densities of the heel bone and the distal end of the radius and ulna were measured before and following flight using photon absorptiometry. Only after 84 days of near weightlessness and only in the heel bone was a significant decrease in bone density observed (Vogel et al. 1977 and Fig. 2). However, no significant loss of urinary hydroxyproline or hydroxylysine glycosides was found throughout the 84-day Skylab flight, indicating no enhancement of bone breakdown (Claus-Walker et al. 1977).

In missions of longer duration, unabated calcium loss could cause not only a decrement in skeletal strength but also perturbations in many physiological systems dependent on calcium for normal function. If complications commonly associated with disuse osteoporosis, such as hypercalcemia, renal stones, and ectopic calcification, occurred during flight, the success of a mission could be severely hampered. Thus, the extent and duration of the calcium loss during flight should be defined prior to extended manned missions.

When the Soviets offered to examine proposals from the USA for possible inclusion among the experiments on the Cosmos 782 biological satellite, investigation of the potential mechanisms involved in changes in bone turnover seemed a logical extension of the Skylab studies. A decreased trabecular mass had been noted in the metaphyses of rats after 22 days onboard the Cosmos 605 spacecraft (Yagodovsky et al. 1976). Bone mass could be lost during spaceflight by decreasing the amount of mineral that goes into bone thence formation and/or increasing the amount of mineral that comes out of bone (bone resorption). The site of the defect should be identified so that meaningful countermeasures could be proposed.

To define further the effects of spaceflight on bone, a proposal to study parameters of periosteal bone formation and endosteal bone resorption in the proximal tibia was submitted and subsequently accepted. In this experiment, young growing rats about 63 days of age at the beginning of flight were injected with a fluorescent bone marker (demeclocycline, a tetracycline derivative) before flight.
fore flight so that bone growth during flight could be measured. A second injection, after flight, was given to quantitate bone growth in the month following flight.

COSMOS BONE RESULTS

The most striking effects were those on bone formation (Morey and Baylink 1978). During flight, rats formed significantly less periosteal bone than did ground control animals. An arrest line found around the periosteum of flight animals suggested that cessation of bone growth occurred during spaceflight. Bone formation in these rats probably stopped sometime after the 11th day of near weightlessness since the total bone mass formed during the 19.5-day flight was laid down by the ground control rats in approximately 11 days. Bone formation was apparently reinitiated within 3 days of reentry, and by 26 days after flight the flight rats showed a significant increase in bone formation rate as compared with vivarium controls. No significant changes in bone resorption were observed.

A similar experiment was flown aboard the next Soviet Cosmos biological satellite, 936, which contained both stationary rats and rats on a short-radius centrifuge. Data from this experiment were virtually identical with those from Cosmos 782 (Table 1). Moreover, in Cosmos 936, (a) centrifugation at 1-G during flight did not appear to correct the effect in tibia diaphyseal bone formation although it did accelerate recovery of bone mass after return to earth, and (b) no rebound in bone formation occurred in the postflight period. Although bone formation did return to normal if the flight rats were compared with the flight controls (which were similarly confined throughout the flight period) rather than with the vivarium controls.

These data suggest that the primary defect in bone turnover in young, growing rats during spaceflight occurs in calcification and that the deficiency is corrected upon return to earth. They also imply that mechanical loading is essential for normal bone turnover. However, many questions remain unanswered: What caused the decreased formation rate? Was it totally due to removal of mechanical loading or did endocrine, neural, or changes in blood flow also contribute to the response? What was the total skeletal response, i.e., did it differ in nonweight-bearing bones, and did total skeletal mass decrease? Did the formation rate actually cease or merely slow down? What would be the response in adult animals? Such questions could not be addressed in long-duration spaceflight experiments until the latter part of the 1980s.

RAT MODELS: A NEW APPROACH

Responses to the near-weightless environment of orbital spaceflight are virtually impossible to study under terrestrial conditions because a 1-G environment is pervading on earth. About the time proposals were submitted for Cosmos 782, animal modeling, a commonly exploited technique for investigating physiological mechanisms, was proposed for studying spaceflight-associated phenomena: data from spaceflight would be available for comparison to determine the validity of the model system. However, such a model was dependent on the development of a device emulating at least some aspects of weightlessness.

Previous attempts with the rat as a test specimen seemed unsatisfactory: total body immobilization or constraint permitted little or no health-maintaining activity. Requirements for an acceptable system included (a) the ability of the animal to exercise using only the front limbs (in a pulling, but nonweightbearing mode); (b) total unloading of the rear limbs without paralysis; (c) a fluid shift; (d) the ability to eat, drink, and groom as normally as possible; and (e) a less stressful system than those presently existing.

The system, which has evolved over the last three years, is shown in Fig. 3. This model is unique in that the animal is free to move about a 360° arc. The rat can pull himself along the plastic mesh with his front paws to reach food and regular laboratory chow: the rear limbs are totally unloaded but unrestrained. The animal is suspended in a head-down mode to perpetrate a fluid shift similar to that seen during orbital spaceflight.

The rat is attached to the model via a freely rotating fishline swivel on an overhanging horizontal aluminum beam. The beam is fixed to a cantilevered aluminum post by a ball bearing rotating in a horizontal plane. The post can be adjusted vertically to compensate for the animal's size and to keep its rear limbs off the grid.

TABLE 1. Comparison of bone parameters in Cosmos 782 and 936.

<table>
<thead>
<tr>
<th>Group</th>
<th>782 Flight</th>
<th>936 Flight centrifuge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endosteal bone resorption</td>
<td>NS*</td>
<td>NS</td>
</tr>
<tr>
<td>% change medullary area (from control)</td>
<td>(+12%)</td>
<td>(-2%)</td>
</tr>
<tr>
<td>Periosteal bone formation rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flight period</td>
<td>47%</td>
<td>43%</td>
</tr>
<tr>
<td>% decrease from control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postflight period</td>
<td>53%</td>
<td>60%</td>
</tr>
<tr>
<td>% increase over vivarium</td>
<td>No data</td>
<td>NS(2%)</td>
</tr>
<tr>
<td>% increase over flight control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flight time required to form total bone volume at control rate (days)</td>
<td>11.1</td>
<td>11.1</td>
</tr>
</tbody>
</table>

*NS = not significantly different from control values.
A paper clip through the Hexcelite harness at the posterior end provides a system for quickly attaching the rat to or disconnecting it from the model. The paper clip is positioned through the harness so that the animal, when connected to the model, maintains about a 30° head-down tilt to shift fluids, intestines, and organs toward the chest. This innovation was suggested when Soviet scientists reported that head-down tilt in humans simulated weightlessness more closely than did horizontal bedrest.

DATA FROM MODEL AND SPACEFLIGHT

Four experiments with suspension periods of approximately 20 days have been conducted to date; this time-period was chosen because it was similar to the duration of Soviet Cosmos biological satellite missions. Weight gain data from these experiments are shown in Fig. 4. Initial average weights of rats were approximately 125 g in the first two experiments, about 150 g in the third experiment, and just over 200 g in the last session. Suspended rats, regardless of their starting size, lost weight the first 2-4 days on the harness. Then they began to gain weight, and within 7-10 days all suspended groups had at least returned to their initial weight.

During the first two sessions weight-matched controls were used; these animals were found to require less food to attain the same body weight as suspended rats. During the third experiment, when harnessed and control rats received the same amount of food (paired), the controls consistently gained about 20% more weight. In the fourth sessions, ad libitum controls gained the most weight; pair-fed controls gained about 20% more weight than suspended rats, and about 20% less food was required to weight-match control animals to the suspended animals. Thus, the weight-gain data showed that the youngster rat when harnessed, the more weight it gained while suspended, and that suspended rats gained less weight per gram of food consumed than did ground controls.

Table 2 compares weight gain and food consumption data from suspended rats with those from Cosmos rats. When these comparisons were made initially, only data from Cosmos 782 were available. At that time, it became obvious that Cosmos rats, like rats on the model (and humans in space), gained less weight per gram of food consumed than did ground controls. Pair-fed ground controls in Cosmos 782 gained about 20% more weight than did flight rats.

Similar results were predicted for Cosmos 936. However, when the data were received, the results were puzzling. Both flight rats and pair-fed ground controls appeared to have gained weight at about the same rate. Finally, word was received that the feeders had malfunctioned during flight and, unexpectedly, gave the rats more food than anticipated. Recalculation of food consumption with the revised data showed, once again, that flight rats gained less weight per gram of food consumed than did ground controls. Thus, the rat model appeared to be very useful in predicting certain metabolic costs of spaceflight.

Table 3 contains bone measurements from suspended and Cosmos rats. To measure peristeam bone formation rate, flight rats were given tetracycline (a fluorescent bone marker that binds to mineralizing bone) three days before flight because of pretreatment scheduling, but suspended rats were given their injection at the beginning of the experimental period. At the end of each experimental period, rats were either sacrificed or given a second injection of marker.
**TABLE 3.** Cosmos and suspended rat model bone parameters.*

<table>
<thead>
<tr>
<th>Item</th>
<th>Suspended</th>
<th>936</th>
<th>782</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals</td>
<td>6</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Initial body weight (g)</td>
<td>205 ± 11.1</td>
<td>212 ± 5.1</td>
<td>225 ± 8.8</td>
</tr>
<tr>
<td>Periosteal bone formation rate (<em>R</em>&lt;sub&gt; Poe&lt;/sub&gt;) % decrease from:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flight control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pair-fed control</td>
<td>44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight-matched control</td>
<td>37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days required to form total bone volume at:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flight control <em>R</em>&lt;sub&gt; Poe&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pair-fed control <em>R</em>&lt;sub&gt; Poe&lt;/sub&gt;</td>
<td>11.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight-matched <em>R</em>&lt;sub&gt; Poe&lt;/sub&gt;</td>
<td>12.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arrest line length (mm)</td>
<td>2.0 ± 1.1</td>
<td>4.0 ± 1.1</td>
<td>5.3 ± 0.6</td>
</tr>
<tr>
<td>(adjusted for length of control arrest line)</td>
<td>(2.0)</td>
<td>(2.4)</td>
<td>(3.2)</td>
</tr>
<tr>
<td>% of periosteal surface</td>
<td>26</td>
<td>52</td>
<td>73</td>
</tr>
<tr>
<td>(adjusted for control)</td>
<td>(26)</td>
<td>(30)</td>
<td>(44)</td>
</tr>
<tr>
<td>Endosteal bone resorption (medullary area)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% change from:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flight control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pair-fed control</td>
<td>NS(−4)</td>
<td></td>
<td>NS(+12)</td>
</tr>
<tr>
<td>Weight-matched control</td>
<td>NS(+2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Data expressed as mean ± standard deviation.
NS = not significantly different from control values.

Second tetracycline label so that read-
sipation could be monitored. Mineral-
cized cross sections were prepared from
the tibiofibular junction. The total bone
area between the tetracycline label (simi-
lar to a tree ring) and periosteal surface
or between two labels was measured and
divided by the number of days of the ex-
periment to give the rate of periosteal
bone formation during the experiment.
Thus, periosteal formation rate is an av-
average for the total experimental period.

Rats that weighed the most at the be-
ninning of the experiment had the lowest
bone formation rate (205 g = 36.8 × 10<sup>−3</sup>
mm/day, 212 g = 25.5 × 10<sup>−3</sup> mm/day.
225 g = 15.8 × 10<sup>−3</sup> mm/day) suggesting
that bone formation decreased as the
animals matured. Suspension appeared to
inhibit bone formation more dramatic-
ally in older rats. Bone formation in
125-g suspended rats decreased only
about 25% from weight-matched con-
trols, whereas formation in 200-g rats
was retarded about 40%. Also, arrest
lines were not detected in young (125-g)
suspended rats: these lines, which in-
dicate a cessation of formation, were
first noted around the periosteum of
flight rats in Cosmos 782. However,
when older harnessed rats (205 g) were
used, arrest lines were found in all sus-
pended rats.

No arrest lines were found in any con-
trol group in the suspension experi-
ments, although in the flight experimen-
tations indications of arrest lines were visible in
control rats. This discrepancy may have
been due to differences in age, strain of
rats, and environmental factors. When
comparing arrest line lengths between
flight and suspended rats (Table 3), if one
corrects for the arrest line in correspond-
ing control animals, then arrest line
lengths in all groups are very similar.
The older rats had the most extensive ar-
rest lines. If bone formation were to cease completely, one might expect the
arrest line to extend completely around
the periosteal surface. This did not occur in any experimental groups (Table 3).
However, if flight values are corrected for control responses, then the percent
of periosteal surface covered by an
arrest line is very similar to the percent
decrease in periosteal bone formation rate.
If rats cease forming bone during suspen-
sion or flight, the earliest that cessation
could occur would be 11 days: the total
bone volume formed by the suspended
or flight rats would have been accrued by
the control animals in 11 days. This
implies that bone formation was constant
prior to arrest; however, formation prob-
ably decreased gradually rather than
suddenly stopping.

**DATA FROM OTHER LABORATORIES**

Models have been sent to other labora-
tories. Data below have been obtained
by Y. Joseph Musacchia, professor of
Physiology and Biophysics, University of
Louisville, and Vojin P. Popovic, pro-
fessor of Physiology, Emory University.
Musacchia and his collaborators have
concentrated on renal function, water,
and electrolyte balance in suspended rats
(Meiningher et al. 1978). For ease of urine
and fecal collection, the model was mod-
ified so that the animal moved through
only a 180° arc and could not turn around.
In the first 2-3 days of suspension, they
found (a) negative water, sodium, and
potassium balance; (b) decreased water
intake with no corresponding decrease
in urine volume; and (c) increased urinary
excretion rates of urea, ammonia, and 3-
methylhistidine. Within 3-4 days, elec-
trolyte balance became normal. Calcium
balance was positive throughout the sus-
penion period.

Their suspended rats also lost weight
the first 3 days of suspension but then
gained weight. Serum sodium decreased,
urea increased, and potassium, calcium,
and osmolality in the serum were normal
at the end of 7 days of suspension. With
the exception of the calcium data, most
values were very similar to those report-
ed during Skylab.

Popovic has concentrated on cardio-
vascular parameters using chronically
nunnulated rats (Popovic and Popovic
1960. Popovic et al. 1963). His prelimi-
ninary data in suspended rats indicated
a decrease in resting mean arterial blood
pressure (which was reflected in in-
creased right arterial pressure), absence
of diurnal variation in heart rate and
mean arterial blood pressure, and sug-
gestions of increased cardiac output dur-
ing the first 2-3 days on the harness.
These data indicate that a fluid shift is
occurring in suspended rats.

**CONCLUSIONS**

The most important components of the
model system would appear to be un-
loading of the limbs and head-down tilt.
These characteristics were common to
all units, although harness materials and
radii of movement differed between labo-
ratories. Weight-gain data in suspended
rats from all laboratories were com-
parable. Of the data obtained thus far,
the results anticipated from Skylab data
included negative water balance, fluid
shift, negative potassium balance, nega-
tive nitrogen balance, muscle atrophy
with increased catabolism, and increased
metabolic cost. Decreased bone forma-
tion rates from the model were very simi-
lar to those of rats flown in the Cosmos
biological satellites. Initial unanticipated
observations from the model have in-
cluded that (a) older rats responded more
dramatically to suspension (and perhaps
weightlessness) than young rats and (b)
gut absorption of water, calcium, so-
dium, potassium, and possibly other substances was decreased.

Thus, many of the responses noted in suspended animals indicate that the model closely mimics results from rats and man exposed to near-weightlessness during orbital spaceflight. Data from experiments using the model system seemingly will allow preliminary answers to questions posed by spaceflight experiments.

ACKNOWLEDGMENTS

The continued enthusiastic support and program direction of Thorne Halstead helped create, build, and test this model system. Dennis Madrid conceived the first generation of the model; Eric Sabelman was invaluable in designing the later model system. Morgan Bedegrew provided excellent technical assistance. David Baylink was coinvestigator on the Cosmos bone experiments, and his laboratory was responsible for bone histomorphology from both the Cosmos and rat model experiments.

REFERENCES CITED


