Proceedings of the Third Annual Meeting of the IUPS Commission on Gravitational Physiology

Published jointly by the American Physiological Society and the National Aeronautics and Space Administration.

A Supplement to
The Physiologist
Volume 24, Number 6
December 1981

International Union of
Physiological Sciences
Commission on
Gravitational
Physiology

PROCEEDINGS OF THE THIRD ANNUAL MEETING OF THE IUPS COMMISSION ON GRAVITATIONAL PHYSIOLOGY

29 September – 2 October 1981

INNSBRUCK, AUSTRIA

DEDICATION

These proceedings are dedicated to Galileo Galilei in recognition of his founding role in gravitational physiology.

Galileo Galilei was born in Pisa, 15 February 1564, the son of a mathematician. At the age of 17 he entered the university at Pisa to study medicine. However, after a few years his talents and interests in mathematics became apparent, and from the age of 21 he became progressively occupied with studies of mathematics and mechanics. Throughout his life he was broadly engaged in physical science and his contributions transformed the medieval natural philosophy into the modern sciences of physics and astronomy. In 1609 he invented a telescope and undertook astronomic observations which led to his support of the Copernican system of the universe. After 1616, Copernican theory was ruled to be heretical, and Galileo was admonished to cease public support of it. Nevertheless in 1632 he published a critique of the Copernican and the accepted Ptolemaic systems (Dialogue on the Two Chief World Systems) and as a result was formally tried and denounced as a heretic in 1633 and constrained from further publication. In 1835, Galileo's "Dialogue" was removed from the Catholic "Index of Prohibited Books," and currently the original finding against him is under review.

In 1633, Galileo was allowed to return from confinement in Rome to his villa near Florence, where he lived the rest of his life in ill health. Although officially silenced, Galileo's mind remained active and he undertook a study of the structure of matter and the nature of motion – resulting in his final and greatest scientific book "Discourses on Two New Sciences." Significantly, this final work was published (in 1638) outside of Italy.

It is in the "Discourses" that Galileo provided the insights which form the beginnings of gravitational physiology in his appreciation of the role of gravitational loading as a determinant of the morphological characteristics of terrestrial organisms as well as of the proportions of man-made structures of different size. The concepts he so lucidly presented were termed the Principle of Similitude by D'Arcy Thompson, and provide a rigorous basis for the quantitative definition of scale effects in biology and engineering alike.

Galileo died at his villa on 8 January 1642 – the year of Isaac Newton's birth – at the age of 78.

Galileo established experimentation in its role in modern science. His aggressive support of ideas developed through experimentation often brought the antagonism of contemporaries. This willingness to argue earned him the nickname "The Wrangler" during his student days. He was, nevertheless, a friendly inspiration to his students who were numerous and loyal. Galileo tended to be informal, refusing to wear the academic robes common to the medieval faculty. In his personal life he was convivial – even to his final days.

Maximas tibi gratias, o Galileo Galilei, pro illa arte scientiaque agimus quam nobis confisus es!

Asimov, I. Biographical Encyclopedia of Science and Technology (2nd ed.). Garden City NY: Doubleday, 1972.

Clavelin, M. The Natural Philosophy of Galileo (Translated by A.J. Pomerans). Cambridge MA: M.I.T. Press, 1974.

DeHarsanyi, Z. The Stargazer. (Translated by P. Tabor). New York: Putnam's Sons, 1939.

Drake, S. Galileo. New York: Hill and Wang, 1980.

Galilei, G. Dialogue Concerning the Two Chief World Systems. (Translated by S. Drake). Berkeley: University of California Press, 1967.

Galilei, G. Discourses Concerning Two New Sciences. (Translated by S. Drake). Madison: University of Wisconsin Press, 1974.

Thompson, D'A. W. On Growth and Form. Cambridge: University Press, 1917.

PROCEEDINGS OF THE THIRD ANNUAL MEETING
OF THE IUPS COMMISSION ON GRAVITATIONAL PHYSIOLOGY
29 SEPTEMBER - 2 OCTOBER 1981
INNSBRUCK, AUSTRIA

The Third Annual Meeting of the Commission on Gravitational Physiology was held in conjunction with the Fall Joint Meeting of the Deutsche Physiologische Gesellschaft and the Österreichische Physiologische Gesellschaft in Innsbruck, Austria, 29 September - 2 October 1981. The meetings opened with a plenary session in which Dr. Earl H. Wood, USA, delivered the keynote address on "Studies of Acceleration Physiology and Descendants Therefrom."

The Gravitational Physiology activities included a one-day symposium of 13 invited papers on a variety of topics, including results of space flight studies on the effects of weightlessness carried out aboard the USSR Salyut-6 Space Station and Cosmos 1129 Biosatellite. Two days of open sessions were also held, during which 42 voluntary papers were given.

The following pages are presented as the Proceedings of the meeting by arrangement with the American Physiological Society. In the interests of timeliness and economy they are published without editorial review. Financial support for the preparation of the Proceedings has been provided by the USA National Aeronautics and Space Administration, for which the Commission is grateful.

Travel support for many of the participants was made available by the National Aeronautics and Space Administration, the European Space Agency, the Free University of Berlin, and the Eidgenössische Technische Hochschule of Zurich. Very special thanks go to Professor Karl Kirsch of Berlin for providing travel support for several of the participants. We should also like to express appreciation to the Deutsche Physiologische Gesellschaft and the Österreichische Physiologische Gesellschaft for their gracious hospitality and provision of excellent facilities for the meeting.

The Fourth Annual Meeting of the IUPS Commission on Gravitational Physiology will be held in San Diego, California, USA on 10-15 October 1982 in conjunction with the Fall Meeting of the American Physiological Society, at their kind invitation. A Preliminary Announcement giving details appears on the next page, and a welcome to participate is hereby extended to all interested scientists.

H. Bjurstedt, Sweden, Chairman
A. H. Brown, USA
A. Cogoli, Switzerland
P. Dejours, France
O. G. Gazenko, USSR
K. Kirsch, GFR
N. Pace, USA
P. E. Pilet, Switzerland
H. Saiki, Japan
A. H. Smith, USA
O. E. Reynolds, USA, Business Officer

PRELIMINARY ANNOUNCEMENT

IUPS COMMISSION ON GRAVITATIONAL PHYSIOLOGY
FOURTH ANNUAL MEETING
10-15 OCTOBER 1982
SAN DIEGO, CALIFORNIA

The Fourth Annual Meeting of the Commission on Gravitational Physiology of the International Union of Physiological Sciences will be held in San Diego, California, 10-15 October 1982. The meeting will be in conjunction with the Fall Meeting of the American Physiological Society, which has kindly agreed to act as host.

The Commission Meeting will comprise open sessions for slide presentations of contributed papers dealing with the effects on physiological systems of humans, animals, and plants of changes in magnitude or direction of the force environment. Included are the effects of the weightlessness of space flight, of acute and chronic acceleration, of vibration, and of the various forms of simulated weightlessness. Also included is consideration of the role of gravity in the manifestation of scale effects in animals and plants. The Commission Meeting will also comprise a one-day symposium by invited speakers on several topics in gravitational physiology.

It is planned to publish the Proceedings of the 4th Annual Meeting in The Physiologist. As previously, the Proceedings will contain the contributed papers and symposium papers presented at the Meeting.

Your participation in the Commission Meeting is welcomed. If you are interested in the particulars, please complete the form below and send it to Dr. Orr E. Reynolds, Commission Business Officer, by 15 February 1982.

- -

Airmail to: Dr. Orr E. Reynolds, American Physiological Society,
9650 Rockville Pike, Bethesda MD 20014, USA

- -

[ ] I wish further information about the 4th Annual Meeting of the IUPS Commission on Gravitational Physiology to be held in San Diego, California 10-15 October 1982.

[ ] I plan to attend.

[ ] I plan to present a paper at the meeting.

[ ] I cannot attend but wish to be kept on the Commission Mailing List.

Name (Please Print) _____

Title _____

Address _____

_____

_____

TABLE OF CONTENTS

GRAVITY 8

Otto H. Gauer (1909 - 1979)

K.A. Kirsch
Free University of Berlin, 1000 Berlin 33, Germany

Otto H. Gauer, one of the most prominent physiologists working in the field of gravitational and cardiovascular physiology, died in January 1979. All of us, especially the ones who were fortunate in having met him in person and having worked with him are deprived of a fascinating personality and an excellent teacher.

When Otto H. Gauer in 1937 began his experiments in Berlin on gravitational and cardiovascular physiology nobody could forsee that ideas and concepts emerging from this work would not only outlive him but in all likelihood the next generation of physiologists. His ideas and concepts came especially to life during his lectures presenting to students his old x-ray films made in the late thirties as well as the latest results of the American Skylab Missions. Those lectures covered a life-long experience in gravitational physiology—and experience which could only be made in his institute. Everybody in the audience became aware of the fact that here was somebody talking about gravity not only as an abstract physical factor appearing merely within mathematical formulas, but as a very practical tool to be used to analyse the cardiovascular and other systems. He made gravity visible to us in the real sense of the word.

His outstanding contributions to cardiovascular physiology are impressive. From his roentgenographic studies a completely new picture of the low pressure system emerged and the concept of blood volume control was developed. The latter came to bear during the close collaboration with James P. Henry in the years between 1949-1954. The close friendship between them resulted in many excellent articles incorporating the latest developments in this special field until the end of the seventies (1, 2).

In amplification of the dedication of the Proceedings of the First Annual Meeting of the IUPS Commission on Gravitational Physiology, *The Physiologist* 22: Suppl., Dec. 1979.

However, more than all those well known and highly respected contributions Otto H. Gauer himself created an atmosphere of certainty as to the future of space physiology, convincing us that still very much remains to be done in the future. From the very beginning he was sure that manned space flight would be possible at a time when for others this was inconceivable. This is documented best in an article entitled "Man under gravity-free conditions," written in 1950: "there are no experimental data regarding the physiological effects of eliminating gravitation for such a long time. We depend more or less on assumptions and conclusions by analogy." (3) Nevertheless his capability to combine intuition with logic allowed him to predict the physiological effects of zero-gravity on the human body with a precision which is astonishing in light of the results reported from experiments done in space two decades later. He was able to transfer his certainty and optimism to his students, colleagues and even to institutions like the European Space Agency (ESA), convincing all of us that the Europeans can contribute substantially to this field of physiology. In his last years he initiated research programs with concepts and aims which are still being carried out today and in the forthcoming Spacelab Missions. In this respect the presence of Otto H. Gauer remains with us.

REFERENCES

1. Gauer, O.H. and J.P. Henry. 1963. Circulatory basis of fluid volume control. *Physiol. Rev.* 43:423-481.
2. Gauer, O.H. and J.P. Henry. 1976. Neurohormonal control of plasma volume. *Intern. Rev. Physiol.* Cardiovascular Physiology II. Ed. A.C. Guyton and A.W. Cowley. Baltimore, University Park Press, 9:145-190.
3. Gauer, O. and H. Haber. 1950. Man under gravity-free conditions. *German Aviation Medicine World War II.* U.S. Government Printing Office, Vol. 1, chapter VI G, 641-644.

REDUCTION IN RENAL ARTERY BLOOD FLOW IMPEDANCE DURING UPRIGHT TILT IN MAN

J. A. Loeppky, E. R. Greene and M. W. Eldridge

Research Division, Lovelace Medical Foundation,
5400 Gibson Blvd. S.E., Albuquerque, NM 87108 USA

Because of the relative inaccessibility of the renal vascular bed, there is little information on the dynamics of renal artery (RA) blood flow in response to alterations in gravitational stress in man. Renal angiography is too traumatic for research purposes and renal clearance techniques are essentially invalid because alterations in blood pressure and hematocrit with orthostasis affect urine flow and indicator concentrations. Previous studies with clearance methods have suggested that RA blood flow (Q_R) is diminished during orthostasis, although the extent and variability of these changes is open to question (4,6). This study was designed to noninvasively characterize the changes in RA impedance and flow during orthostasis and compare them to changes in the central circulation in an attempt to define the redistribution of pressures and flows occurring in the central and renal vascular beds.

MATERIALS AND METHODS

Four females and 2 males served as subjects (mean age: 29 yr, weight: 58 kg, BSA: 1.68 m^2). A 5.0 MHz real-time, 2-dimensional pulsed Doppler duplex scanner (PDD-ATL, model Mark V) was utilized to obtain right or left RA diameter (d), blood velocity (v) waveforms and the Doppler angle before, during, and after 20 min of passive upright tilt (UP) on a tilt- table at 60°. The acoustic window for the transducer scanhead was about 2 to 3 cm caudal to the xyphoid process. Mean v for 5 cardiac cycles was obtained by integrating the hardcopy waveforms of the spatially-averaged v with a digitizer and microcomputer. The detailed methods and validation of determining Q_R by this technique have been described elsewhere (2). A sample image and waveform before and during UP in one subject are given in Fig. 1. Stroke volume and cardiac output (Q_C) were also obtained noninvasively from the ascending aorta with the PDD as described previously (3) within a minute of the RA measurements. Mean brachial artery blood pressure obtained at similar times by sphygmomanometer was used with Q_C to calculate total peripheral resistance (TPR) in mmHg/ml/sec. The ratio of peak to mean velocity was calculated from both aortic (P/M_C) and RA (P/M_R) waveforms. This value has been shown to be directly related to downstream impedance (1). The average value for d was 0.5 cm and did not change with UP so the mean value for each subject was used for all measurements during the run.

RESULTS AND DISCUSSION

The mean values ±SE of the 6 subjects are summarized in Table 1. The test for one subject was terminated after 15.5 min because of impeding syncope. Heart rate (HR) rose 38% after 15 min and Q_C fell 21% as stroke volume declined by 43% (50% after 20 min). The absolute values for Q_C are obviously below those reported with other techniques; however, the relative changes are probably valid (3). These values returned to near baseline after 5 min of recovery. The compensatory vasoconstrictor response to UP is reflected in the 55% increase in TPR after 20 min, which then declined in recovery. The excellent correlation between the rise in P/M_C values and TPR with UP is shown in Fig. 2. This clearly demonstrates that relative changes in impedance in the total peripheral circulation can be accurately predicted by this analysis of aortic waveforms. If this correlation holds in the renal vascular bed then gravitational stress must result in a reduction in renal vascular impedance indicative of arteriolar dilation, since P/M_R was reduced (Table 1). P/M_R was significantly lower from 10 to 20 min of UP ($p < .05$) and was still reduced 5 min after return to supine. The resting baseline values for Q_R are in the range of those reported by other techniques, i.e., about 20% of Q_C for both renal arteries.

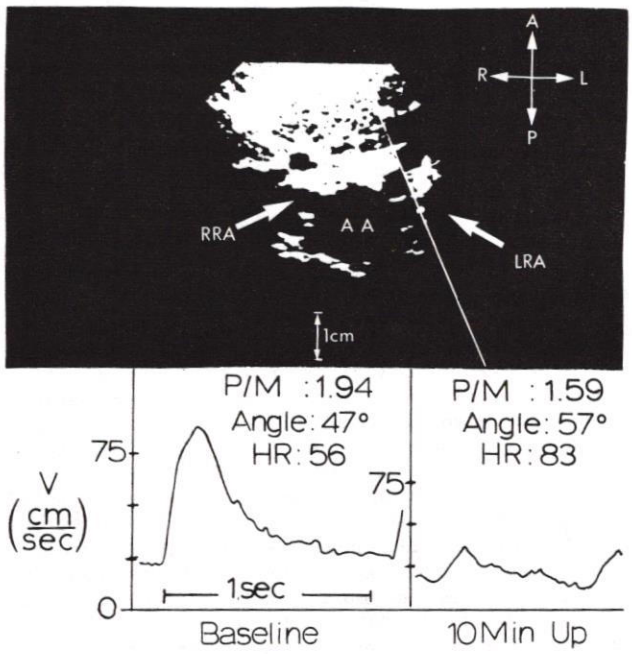


FIGURE 1. Image and waveforms (AA:abdominal aorta)

TABLE 1. Responses to 20 min UP at 60°

Time min	n	HR per min	$\dot{Q}_C$ L/min	TPR mmHg ml/sec	P/M_C	$\dot{Q}_R$ ml/min	$\dot{Q}_R/\dot{Q}_C$	P/M_R
Base-line	6	55 ±3	3.75 ±0.68	1.62 ±0.28	5.69 ±0.58	415 ±47	0.13 ±0.03	1.77 ±0.06
5	6	69 ±4	3.44 ±0.51	1.85 ±0.36	6.06 ±0.73	339 ±100	0.11 ±0.03	1.72 ±0.14
10	6	72 ±5	3.13 ±0.54	2.09 ±0.32	6.22 ±0.64	356 ±98	0.12 ±0.02	1.56 ±0.07
15	6	76 ±6	2.95 ±0.47	2.17 ±0.33	6.38 ±0.74	383 ±111	0.14 ±0.03	1.62 ±0.06
20	5	76 ±6	2.57 ±0.39	2.51 ±0.39	7.12 ±0.68	417 ±90	0.16 ±0.02	1.52 ±0.06
1	6	63 ±6	3.06 ±0.39	1.89 ±0.27	6.41 ±0.64	355 ±56	0.12 ±0.02	1.62 ±0.06
5	6	54 ±3	3.59 ±0.70	1.74 ±0.36	5.59 ±0.79	419 ±105	0.13 ±0.03	1.62 ±0.04

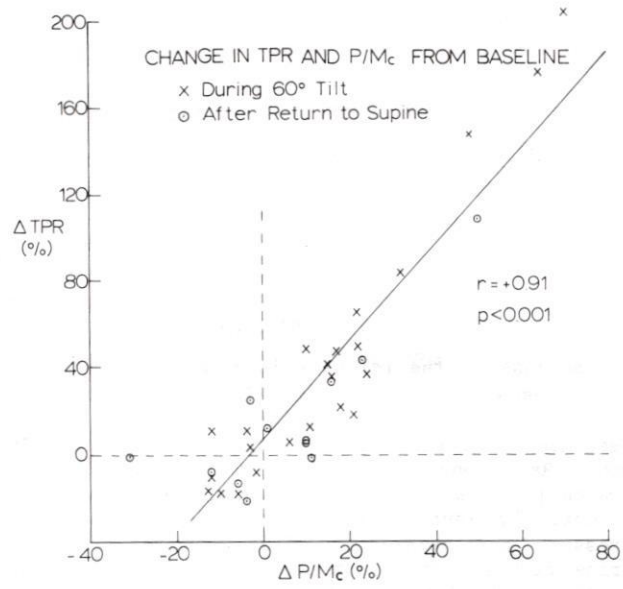


FIGURE 2

After 5 min of UP, $\dot{Q}_R$ had fallen 18%. This average decline in $\dot{Q}_R$ was greatly attenuated by the 80% rise in one subject whereas the other 5 showed an average drop of 39%. Thereafter, the mean flow returned to the baseline values during UP because of the increase noted in 2 other subjects. In 3 subjects, including the one with poor orthostatic tolerance, $\dot{Q}_R$ was reduced by an average of 52% during the last 3 UP measurements. The large SE for $\dot{Q}_R$ reflects the variation between subjects and undoubtedly some measurement error. The determination of the Doppler angle from the freeze-frame image (Fig. 1) is the most likely source of error; at best it can be determined to within ±5°. For the mean angle for these measurements (58°) a ±5° error would result in v and $\dot{Q}_R$ being in error by ±14%. The fact that the coefficient of variation (CV) for P/M_R (which is angle independent) was only 10%, as compared to 55% for $\dot{Q}_R$, indicates a true variability in response to orthostasis, especially since the CV for baseline $\dot{Q}_R$ was only 28%. The ratio $\dot{Q}_R/\dot{Q}_C$ did not change appreciably with UP, decreasing in 3 subjects and increasing in 2. The average UP value was identical with the baseline value (0.13). This shows that the relative reduction in $\dot{Q}_R$ was the same as that for $\dot{Q}_C$ and that no striking redistribution of flow took place in the renal vascular bed in relation to total central blood flow. These results suggest that the renal vascular bed maintains its blood flow by vasodilation during orthostasis while the net result of all vascular beds is a vasoconstriction. The calculated RA pressure (from $\dot{Q}_R$ and P/M_R) showed an increase in those 2 subjects who increased $\dot{Q}_R$ and a decrease in the other 4, the average drop for all UP measurements being 15%. Since average blood pressure (brachial artery) increased 8% during UP, this implies a reduction of pressure distinctive to the renal vasculature.

It has been suggested that the renin-angiotensin mechanism is a significant factor in the maintenance of circulatory homeostasis during orthostatic stress (5). Our results suggest that if such a release of renin occurs during orthostasis, it cannot be attributed to an increase in RA pressure since this declined, but could still be triggered by the early reduction in $\dot{Q}_R$. Although no cause-and-effect can be proven with these data, there was a negative correlation between the reduction in $\dot{Q}_R$ and the increase in TPR ($r = -0.56$) and if the subject who experienced near-syncope (and had no increase in TPR) was eliminated, the correlation improved to $r = -0.81$ ($p < 0.10$). In spite of the potential errors in the measurements reported here for $\dot{Q}_R$, the data indicate that the renal vasculature reacts by dilating under orthostatic stress and that large variations exist in the response of $\dot{Q}_R$ between subjects.

REFERENCES

(1) Beasley, M., J. Blau, R. Gosling. Changes in internal carotid artery flow velocities with cerebral vasodilation and constriction. Stroke. 10:331-343, 1979.

(2) Greene, E., M. Venters, P. Avasthi, R. Conn, R. Jahnke. Noninvasive characterization of renal artery blood flow. Kidney Int. 19: (In press), 1981.

(3) Loeppky, J., E. Greene, D. Hoekenga, A. Caprihan, U. Luft. Beat-by-beat stroke volume assessment by pulsed Doppler in upright and supine exercise. J. Appl. Physiol. 50: 1173-1182, 1981.

(4) Molzahn, M., T. Dissmann, S. Halim, F. Lohmann, W. Oelkers. Orthostatic changes of haemodynamics, renal function, plasma catecholamines and plasma renin concentration in normal and hypertensive man. Clin. Science. 42:209-222, 1972.

(5) Oparil, S., C. Vassaux, C. Sanders, E. Haber. Role of renin in acute postural homeostatis. Circulation. 41:89-95, 1970.

(6) Wolthius, R., S. Bergman, A. Nicogossian. Physiological effects of locally applied reduced pressure in man. Physiol. Rev. 54: 566-595, 1974.

RELEVANCE OF ALDOSTERONE ON CIRCULATORY, RENAL AND BLOOD REACTIONS DURING SIMULATED WEIGHTLESSNESS AND ON AEROBIC METABOLIC RATE.

W. Skipka and J. Stegemann

Physiologisches Institut, Deutsche Sporthochschule Köln,
D-5000 Cologne, Carl-Diem-Weg, Federal Republic of Germany

In order to determine the relevance of the decrement in aldosterone level during water immersion (IM) with respect to circulatory, renal and blood reactions, 4h IM experiments were conducted in which aldosterone was substituted by injections of Aldocorten at the beginning and after 2 h of IM. The results are compared with controls (IM without aldosterone injection). Blood pressure was significantly less reduced without any aldosterone specific influence on heart rate. Aldosterone-induced deviations of renal function had been too small to change blood volume and its' constituents essentially. Only red cell 2,3-DPG concentration increased significantly in the opposite direction to that of the controls. Additional experiments revealed that aldosterone significantly augmented oxygen uptake during work load. This indicates that the IM-induced decrease of performance capacity can be due to the concomitant aldosterone decrement.

INTRODUCTION

During several hours of simulated weightlessness (head-out water immersion = IM) a decrease of approximately 50 % aldosterone secretion has been observed by several authors (5,10). At the same time IM caused alterations in circulatory behaviour, renal function and some blood values (2,4,6,13). The aim of our investigation was to determine the influence of lowered aldosterone level on these parameters.

METHODS

A series of IM experiments lasting 4h were conducted with 12 healthy male subjects (aged 23-33 years) which lay in a semi-recumbent position in a water bath of 35.5°C. At the beginning and after 2h of IM, aldosterone was substituted by subcutaneous injections of 0.5 mg Aldocorten® (aldosterone experiments = AE). The results were compared with experiments without aldosterone substitution (control experiments = CE).

RESULTS AND DISCUSSION

Fig. 1 demonstrates that blood pressure decreased during the early stages of IM while the heart rate increased. Aldosterone injections caused a significantly lower reduction in blood pressure (systolic: $p<0.001$; diastolic: $p<0.05$). There was no indication of there existing any specific influence on heart rate as a consequence of aldosterone. Reasons for the aldosterone-induced alteration in blood pressure behaviour may be (i) a result of possible inotropic effect of aldosterone on myocardium (12) and (ii) the fact, that aldosterone enhances the blood pressure action of angiotensin II and the catecholamines (7,8). A protective effect for orthostatic stability after simulated weightlessness conditions might be effected.

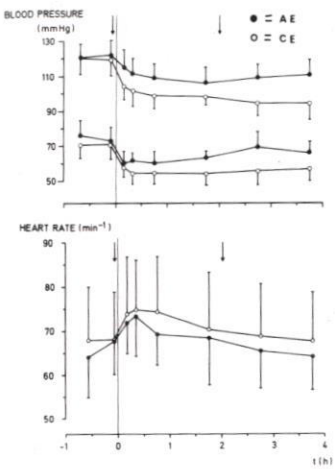


Figure 1:
Blood pressure and heart rate before and during immersion with (●) and without (o) aldosterone substitution. Arrows mark the time of aldosterone injections. (Means ± SE).

Fig. 2 shows a synopsis of the water-balance. By recording both components for the diuresis (C_{osm} and C_{H2O}) it can be seen that the IM-induced diuresis ($\dot{V}_U$) is an osmotic one even in the AE. No specific aldosterone effect on the osmolar clearance (C_{osm}) was observed. While free-water clearance (C_{H2O}) was reduced significantly in the CE ($p<0.05$) no systematic changes were revealed after aldosterone application. The small augmentation of diuresis in the AE was mainly due to the missing decrement of C_{H2O} in this group. Glomerular filtration rate (GFR) rose significantly greater

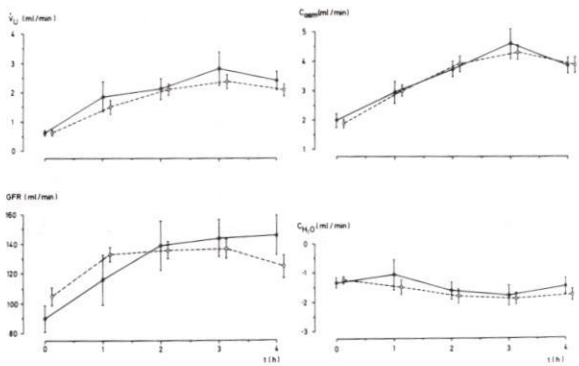


Figure 2: Urine flow ($\dot{V}_U$), glomerular filtration rate (GFR), free water (C_{H2O}) and osmolar clearance (C_{osm}) before and during immersion. Symbols as in Fig. 1. The values reflect measurements of the sample period of the preceding hour.

and more prolonged after aldosterone injection ($p<0.05$). In spite of the fact that the concomitant natriuresis in the AE was much less pronounced compared to the controls (see Fig.3), C_{osm} was not diminished due to the GFR effects. The reduction in sodium excretion caused by aldosterone treatment started in the second h of IM and was accompanied by a significantly smaller increase in urinary pH-values, while kaliuresis was marked from the third h onwards.

Blood samples, drawn after 2 and 4h, revealed that aldosterone did not act significantly on the IM-induced changes of the following blood and plasma values: Haematocrit, haemoglobin, pH, plasma protein as well as sodium and potassium concentration and osmolality. This indicates that the aldosterone-induced changes of renal function during 4h IM did not essentially act on blood volume and its constituents.

In earlier investigations we demonstrated that during IM red cell 2,3-diphosphoglycerate (2,3-DPG) concentration and aldosterone excretion showed a similar patterned decrease (3). Fig. 4 depicts the results of the present experiments: During IM with aldosterone application 2,3-DPG increased by 0.31 ± 0.10 ($\bar{x}\pm s_{\bar{x}}$) mmol/l erythrocytes, while it was decreased during normal IM by 0.38 ± 0.14 (both: $p<0.01$). This findings support the hypothesis that aldosterone acts on 2,3-DPG metabolism and influences as a result, the oxygen dissociation curve of haemoglobin as observed by other authors (1). Further experiments without immersion revealed that aldosterone significantly increased oxygen uptake during submaximal and maximal work-load by approximately 10 % (9). Obviously, a close relationship

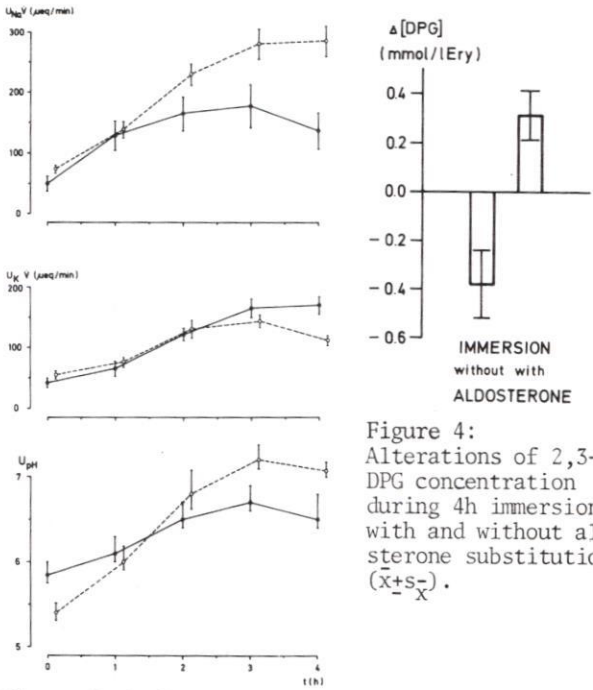


Figure 4: Alterations of 2,3-DPG concentration during 4h immersion with and without aldosterone substitution. ($\bar{x}\pm s_{\bar{x}}$).

Figure 3: Sodium and potassium excretion and urinary pH values before and during immersion. Symbols and comments as in Fig. 1 and 2.

exists between performance capacity and aldosterone level; this indicates that the IM-induced decrease of performance capacity (11) can be explained by the concomitant aldosterone decrement.

References

1. Bauer, Ch., A.-M.Rathschlag-Schaefer: The influence of aldosterone and cortisol on oxygen affinity and cation concentration of the blood. Resp.Physiol. 5,360-370,1968
2. Böning,D., H.-V.Ulmer, U.Meier, J.Stegemann: Effects of a multi-hour immersion on trained and untrained subjects: II. Blood protein and electrolyte concentrations. Aerosp.Med. 43,415-418,1972
3. Böning,D., U.Meier, W.Skipka, W.R.Külpmann, K.A.Meurer: Some evidence for aldosterone action on 2,3-diphosphoglycerate level in human red cells. Metabolism 25,9-14,1976
4. Böning,D., W.Skipka: Renal blood volume regulation in trained and untrained subjects during immersion. Eur.J.Appl.Physiol. 42,247-254,1979
5. Epstein,M., T.Saruta: Effect of water immersion on renin-aldosterone and renal sodium handling in normal man. J.Appl.Physiol. 31,368-374,1971
6. Epstein,M.: Cardiovascular and renal effects of head-out water immersion in man. Circ.Res. 39,619-628,1976
7. Morandini,G., M.Spanedda, L.Spanedda: Ulteriori osservazioni sul potenziamento cortisonico e aldosteronico dell'azione dell'angiotensina nell'uomo. (Further observations on the cortisonic and aldosterone potentiation of the action of angiotensin in man.) Minverva Med. 58,1552-1556,1967
8. Ross,E.J.: Functional relationship between adrenal medullary and cortical hormones in man. Quart.J.Med. 30,285-296,1961
9. Skipka,W., L.Schöning, J.Stegemann, H.Vetter: Increase of $V'o_2max$ induced by aldosterone. Pflügers Arch. 377,R 34,1978
10. Skipka,W., D.Böning, K.A.Deck, W.R.Külpmann, K.A.Meurer: Reduced aldosterone and sodium excretion in endurance-trained athletes before and during immersion. Eur.J.Appl.Physiol. 42,255-261,1979
11. Stegemann,J., H.D.Framing, M.Schiefeling: Der Einfluß einer 6-stündigen Immersion in thermoindifferentem Wasser auf die Regulation des Kreislaufs und die Leistungsfähigkeit bei Trainierten und Untrainierten. Pflügers Arch. 312, 129-138, 1969
12. Tanz,R.D.: Studies on the inotropic action of aldosterone on isolated cardiac tissue preparations; including the effects of pH, ouabain and SC-8109. J.Pharmacol.exptl.Therap. 135,71-78,1962
13. Ulmer,H.-V., D.Böning, J.Stegemann, U.Meier, W.Skipka: Pulsfrequenz, Blutdruck, Blutvolumen und Sauerstoffaufnahme von Ausdauer-Sportlern und Nichtsportlern während Immersion in Wasser. Z.Kreisl.-Forsch. 61, 934-946, 1972

ERYTHROPOIETIC EFFECTS OF SPACEFLIGHT RE-EVALUATED

C.D.R. Dunn[1], P.C. Johnson[2] and J.I. Leonard[3]

[1]Northrop Services Inc., P.O. Box 34416, Houston, TX 77034 and Division of Experimental Biology, Baylor College of Medicine, 1200 Moursund Avenue, Houston, TX 77030; [2]Medical Research Branch, Lyndon B. Johnson Space Center, National Aeronautics and Space Administration, Houston, TX 77058; [3]Management and Technical Services Co., 1050 Bay Area Blvd., Houston, TX 77058.

ABSTRACT: Animal and computer models have been used to investigate the hypothesis that dehydration, by causing an increase in hematocrit due to a relative increase in red cell mass (RCM), could explain the suppression of red blood cell (RBC) production which results in the "anemia" of spaceflight. Instead the data from both models suggest that the primary cause of the erythroid suppression was, in some way, related to the loss of body weight while the elevated hematocrit was considered of minor importance. With this data base a re-evaluation of the Apollo and Skylab results was undertaken from the point of view that decreases in RCM might be linked to decreases in body weight. The analyses suggest that the "anemia" of spaceflight might be due to an adaptation to a lower body weight and that it may be prevented by mechanisms, such as exercise, designed to increase tissue oxygen demand and/or maintain lean body mass (LBM).

A post-flight decrease in the RCM has been one of the more consistent but least expected biomedical effects of spaceflight (1,2). Although there is some evidence to suggest a small increase in the rate of destruction of mature RBC during flight (2,3), most of the available information suggests that the primary cause of the, so-called, "anemia" of spaceflight is suppression of cell production at least on the longer missions. Several years ago we set out to test the hypothesis that suppression of RBC production during spaceflight followed as a consequence of the fluid shifts that occur in 0g. According to this theory the cephalic shift of body fluids (2) results in a water loss secondary to either an active diuresis, an increase in insensible water loss and/or temporary cessation of the thirst reflex. Whatever the pathway of fluid loss, a consequence of this phenomenon appears to be a diminished plasma volume and an elevated hematocrit. This relative increase in RCM was proposed to suppress RBC production by a mechanism similar to that which operates after an absolute increase in RCM after, for example, hypertransfusion. After RBC transfusion, suppression of erythropoiesis occurs through decreases in serum titers of erythropoietin (Ep) and of the more mature erythroid progenitor cells in the bone marrow and spleen (4). Recovery of RBC production occurs when the hematocrit falls to normal due to cell senescence. A similar "recovery" mechanism (normal hematocrit but at a reduced plasma volume and RCM) was invoked to explain the Skylab data where the decrease in RCM was smallest on the longer mission.

In mice, complete deprivation of water resulted in a plasma volume decrease and, within 24 hours, erythropoiesis was suppressed despite normal serum Ep titers. This suppression appeared to be mediated through an impaired ability of the bone marrow to respond to Ep (5). By the 3rd and 4th days of dehydration, RBC production was almost totally inhibited, serum Ep titers were below the detection limit of the assay, all classes of erythroid progenitor cells were greatly reduced, the RCM was below normal and the mice had lost over one-third of their body weight (5). A comparison of these changes with those occurring in mice with an increased hematocrit secondary to an absolute increase in RCM (4), showed several important qualitative and quantitative differences indicating that the hematocrit increases alone were not sufficient to explain the erythrosuppression in dehydrated mice. Further studies (5-7) suggest that the primary cause of the "anemia" in dehydrated animals and man is related to the concomitant weight loss. Computer simulations (5) of the relative inportance of food and water balance also confirm that erythropoiesis is more sensitive to metabolic deficits than to hematocrit increases due to fluid restriction.

Since there has been concern about man's energy status during spaceflight (8,9), a re-evaluation of the data from the Apollo and Skylab missions was undertaken to determine if there was any evidence to link changes in body weight (10,11) to decreases in RCM. In both the Apollo and Skylab crews the physiologically well-established significant correlation between LBM and RCM was observed both preflight and postflight. However, postflight the relationship was shifted towards a lower LBM and smaller RCM (Figure 1) and the decrements in RCM correlated directly with the decrements in body weight (Figure 2). Further investigations, based on published observations, indicated that caloric intake (8) and exercise level (12) should also be considered as potential causes for the RCM decreases seen during the Skylab missions. Multi-variate regression analysis yielded the equation;

$$\Delta RCM = 11.0(\Delta DIET) + 0.0004(\Delta LBM) - 169.1(EXERCISE) - 325.9$$

which shows a highly significant ($r = 0.8063$, $P<.001$) correlation between the decrease in RCM and the decreases in caloric intake and LBM. Also the higher the exercise level, the smaller the decrease in RCM. In other words, changes in diet, LBM and

exercise level may all interact in a complex way to affect the RCM. The loss of LBM is the major factor in this correlation.

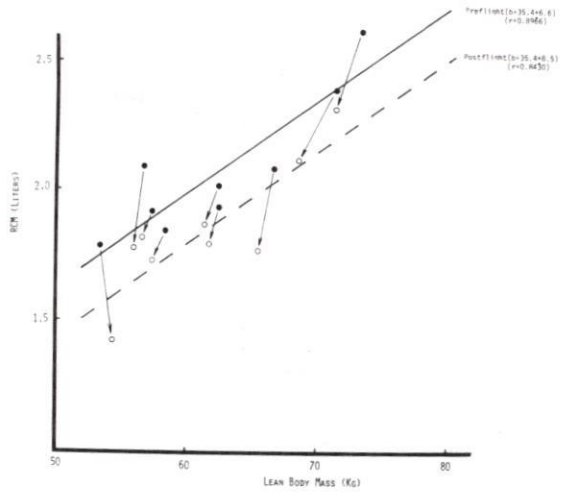


FIGURE 1: Correlations between red cell mass (RCM) and lean body mass (LBM) in the nine crew members before (solid symbols and line) and after (open symbols and broken line) the three Skylab missions.

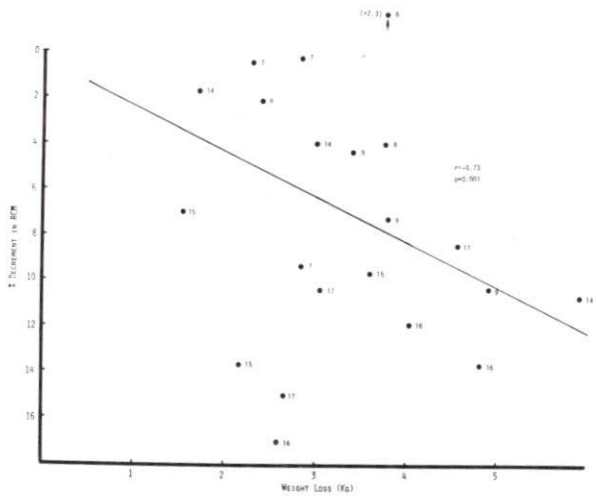


FIGURE 2: The relationship between changes in red cell mass (RCM) and changes in body weight in the crew members of the Apollo missions. (Figures refer to mission number).

These analyses suggest, therefore, that the "anemia" of spaceflight may represent an adaptation to a lower body weight and might, at least partially, be prevented by techniques such as exercise which either help to maintain LBM and/or directly or indirectly increase tissue oxygen demand. Although this theory, based on standard physiological principles and supported by a variety of experimental, clinical and modelling studies, can explain the variations in RCM seen between the Skylab missions without invoking the concept of erythroid regeneration (for which there was little evidence) many factors need further study. Most importantly, it is not clear whether the changes in LBM directly result from reduced food intake or whether there are more subtle metabolic effects of spaceflight leading to a redirection of energy deposition from muscle to fat. Nor is it known which forms of exercise might be most efficient at maintaining the RCM.

REFERENCES:

1. KIMZEY, S.L., C.L. FISCHER, P.C. JOHNSON, S.E. RITZMANN, AND C.E. MENGEL. Hematology and immunology studies. In: Biomedical Results of Apollo, edited by R.S. Johnston, L.F. Dietlein and C.A. Berry. Washington, DC: NASA, 1975, Publ. NASA SP-368, p. 197-226.

2. KIMZEY, S.L. Hematology and immunology studies. In: Biomedical Results from Skylab, edited by R.S. Johnston and L.F. Dietlein. Washington, DC: NASA, 1977, Publ. NASA SP-377, p. 249-282.

3. LEON, H.A., AND S.A. LANDAW. Effect of weightlessness and centrifugation (1 x G) on erythrocyte survival in rats subjected to prolonged spaceflight. In: Final Reports of U.S. Experiments Flown on the Soviet Satellite Cosmos 936, edited by S.N. Rosenzweig and K.A. Souza, NASA/Ames Research Center CA, 1978, p. 60-77.

4. DUNN, C.D.R. Investigations into the in vitro sensitivity to erythropoietin of haematopoietic tissue from ex-hypoxic, erythrocytotic mice. Br. J. Haemat. 44: 619-626, 1980.

5. DUNN, C.D.R., J.I. LEONARD, AND S.L KIMZEY. Interactions of animal and computer models in investigations of the "anemia" of spaceflight. Aviat. Space Environ. Med. (in press).

6. GIGLIO, J.M., R.M. ALIPPI, A.C. BARCELO, AND C.E. BOZZINI. Mechanism of the decreased erythropoiesis in the water-deprived rat. Br. J. Haemat. 42: 93-99, 1979.

7. HOWARTH, C., I. CHANARIN, A. JANOWSKA-WIECZOREK, AND J.A.F. NAPIER. Familial erythrocytosis. Scand. J. Haemat. 23: 217-222, 1979.

8. RAMBAUT, P.C., C.S. LEACH, AND J.I. LEONARD. Observations on energy balance in man during spaceflight. Am. J. Physiol. 233: R208-R212, 1977.

9. RAMBAUT, P.C., M.C. SMITH, AND H.O. WHEELER. Nutritional studies. In: Biomedical Results of Apollo, edited by R.S. Johnston, L.F. Dietlein and C.A. Berry. Washington, DC: NASA, 1975, Publ. NASA SP-368, p. 277-302.

10. THORNTON, W.E., AND J. ORD. Physiological mass measurements in Skylab. In: Biomedical Results from Skylab, edited by R.S. Johnston and L.F. Dietlein. Washington, DC: NASA, 1977, Publ. NASA SP-377, p. 175-182.

11. WEBB, P. Weight loss in men in space. Science. 155: 558-560, 1967.

12. THORNTON, W.E., AND J.A. RUMMEL. Muscular deconditioning and its prevention in space flight. In: Biomedical Results from Skylab, edited by R.S. Johnston and L.F. Dietlein. Washington, DC: NASA, 1977, Publ. NASA SP-377, p. 191-197.

ACKNOWLEDGMENTS:
Supported by contracts, NAS 9-14523, 9-15425, 9-15487 and 9-16180 with The National Aeronautics & Space Administration.

RELATION BETWEEN SENSORIC AND MOTORIC FUNCTION OF WISTAR RATS AFTER SPACE FLIGHT BY BIOSATELLITE COSMOS 1129

M. Poppei, K. Hecht, V.S. Oganov, V.Ja. Klimovitzky,
Th. Schlegel, D. Sass, E. Wachtel, V.S. Magedov

Department of Neuropathophysiology, Humboldt-University Berlin, GDR

Institute of biomedical Problems, Moscow, USSR

Introduction

Readaptation to gravity following a several weeks space flight ever and again raises new questions. It's known that distinct changes in the motor system will occur following a several weeks space flight /1/. The above mentioned changes have been reversible: Twentyfive days after termination of space flight the estimated changes had become similar to those of the controls. However, until now investigation in single muscles or muscles complexes have been performed only. Firstly, in this context the questions arose by what way this structural and functional impairment of the motor system after a space flight will affect the entire coordinative motor behaviour, for example the speed of performance of a locomotor reaction. Secondly, in connection with that problem the question has been proposed whether the changes in the motor system caused by weightlessness influence the central nervous reaction /2/. As it is known, "the function of the effectoric motor system will be induced by each strong stimulation, that means the function of neocortex being extensively dependent on the functional activity of its muscular partner". Thirdly, in connection with studies performed in our laboratory /3/ the question arose whether motor and central nervous reaction times also during the post flight readaptation period could be influenced by the circadian sensitivity phase of motility.

Method

The proposed questions have been worked up by using a hardly extinguishable locomotor avoidance reflex /4/ readily elaborated in the animals before space flight. By this method (fig. 1) it had been possible to determine the following two parameters by automatic timing clock.

1. The central nervous reaction time (CRT), the time lasting from the beginning of the conditioned stimulation to the start of the loccomotor reaction.
2. The motor reaction time (MRT), the time from the beginning of the lo comotor reaction to lever press. The fully trained rats started in the biosatellite Cosmos 1129 for a 18 1/2 days space flight. Five, ten, fifteen and twenty days after landing the CRT and MRT at maximum as well at minimum of the animals motor activity were measured. Three groups of animals have been studied: Space flight-group (bios), Synchronous-group (bios), Control-group (vivarium).

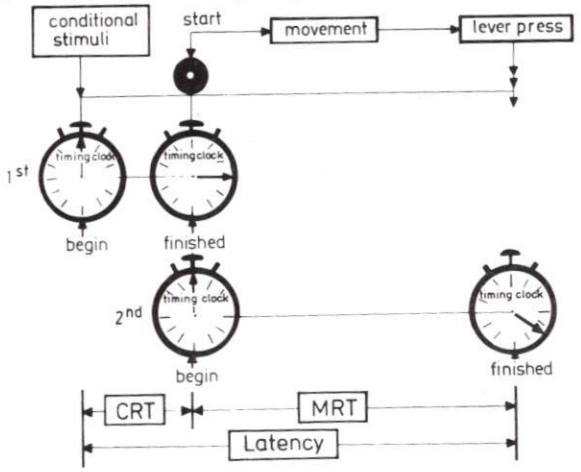


Fig. 1

Results

At minimum of motor activity (fig.2), (i.e. during light phase) the control-group showed relatively constant motor and central nervous reaction times on all days of investigation. As demonstrated by a correlation coefficient of o.88 accompanied by a low degree of variation, there was a strong correlation between the two parameters.

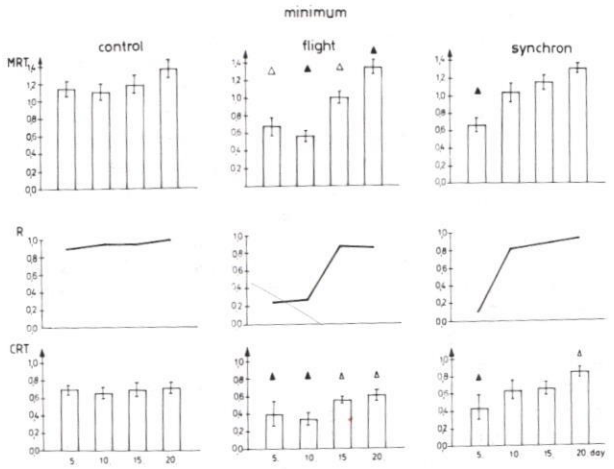


Fig. 2

In the space-flight-group very short motor and central nervous reaction times on the 5th and 1oth day after landing under light-phase conditions have been observed. The correlation coefficient was strong reduced (r=o.23). However, on the 15th day after landing the parameters had approximated to those of the control-group. On the 5th day after maintenance under simulated space-flight conditions shortened MRT and CRT as well as a low correlation coefficient have been recorded in the so called synchronous-group. From the 1oth day after space-flight simulation reaction times had become quite normally in the synchronous-group.
At maximum of motor activity (fig.3),(i.e. during dark-phase) there were about analogous changes with regard to reaction times, but a qualitative difference in comparison to the values measured during light-phase was to be seen. Although correlation between MRT and CRT already in the controls had been smaller at maximum than at minimum of activity, there was in the space-flight-group as well as in the synchronous-group a longer readaption periode.

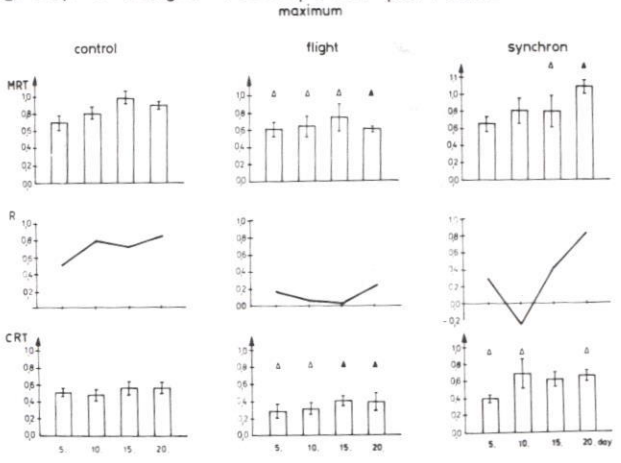


Fig. 3

Discussion

The obtained results show that the changes of muscle structure being recorded after a 18 1/2 days space-flight are reflected by changed properties of an elaborated motor reaction too. The distinctly shortened motor reation times indicated a motor hyperactivity are in agreement with the increased metabolism of muscles as a result of overcoming the state of weightlessness and as an indication for stress reaction as it in a similar way was found by us in former studies in connection with hypokinetic stress /5/. Motor hyperactivity has been reversible which likewise goes parallel to morphological and biochemical processes. The recorded motor hyperactivity has its pendant in the measured central nervous reaction, for the reaction times concerning the sensoric functions have been appropriately shorted. Thus, also following the state of weightlessness the close correlation between central nervous and motor functions could be proved.In connection with the results of other authors regarding stress /6,7/ we believe that there is a process of interaction characteristed by the relation afference - reafference on the one hand and by specific reactions (motor system→CNS) as well as unspecific reactions (CNS↔motorsystem) of stress on the other hand. The difference between the space-flight-group and the synchronous-group regarding the injury the motor as well as of the central nervous function could be due to the different intensities and numbers of stressors being involved. In comparison to the space-flight-group in the synchronous-group the loading was reduced by one stressor namely weightlessness. As it could be demonstrated the observed changes seem to depend on the circadian sensitivity phases. At maximum of motor activity there was a distingly diminished degree of coupling between the MRT and CRT throughout nearly the whole investigation period of the flight-group. It may be concluded that there still exists a latent regulatory instability. The process of readaptation to Earth gravity following a space-flight obviously is such a complex one that only multifactorial analyses combined with continous measuring over the whole investigation period may lead to a better insight in this scientific field.

References

1. Oganov, V.S., S.A. Skuratova, A.I. Potapov, M.A. Shirvinskaya: The Physiologist 23/6 (suppl) (198o) S 16-S 21
2. Anochin, P.K.: Das funktionelle System als Grundlage der physiologischen Architektur des Verhaltensaktes. Abh. aus dem Gebiete der Hirnforschung und Verhaltensphysiologie. Bd.1, VEB Gustav Fischer Verlag Jena 1967
3. Hecht, K., J.P. Ljowschina, Th. Schlegel, M. Poppei, K. Treptow: Acta biol. med. germ. 33 (1976) 973-982
4. Hecht, K., K. Treptow, S. Choinowski, M. Peschel: Die raumzeitliche Organisation der Reiz-Reaktions-Beziehungen bedingt-reflektorischer Prozesse. Abh. aus dem Gebiete der Hirnforschung und Verhaltensphysiologie Bd.5 VEB Gustav Fischer Verlag Jena 1972 S.12 ff.
5. Poppei, M.: Chronischer Stress in Abhängigkeit von Zuständen des Organismus und von Umweltkonstellationen(Tierexperimentelle Studien). Doktordissertation (B) Berlin 198o
6. Selye, H.: J. of Medicine 75/12 (1975) 3139-3145
7. Levi, L.: Stress and Distress in Response to physiological Stimuli. Acta med. Scand. (suppl.) 528 1972

ATROPHY OF RAT SKELETAL MUSCLES IN SIMULATED WEIGHTLESSNESS

D.D. Feller, H.S. Ginoza, and E.R. Morey

Biomedical Research Division
NASA-Ames Research Center
Moffett Field, California USA

ABSTRACT

A hypokinetic rat model was used for elucidation of the mechanism of skeletal muscle wasting which occurs in weightlessness. Rats were suspended from a back-harness with the head tilted downward and the hind limbs totally unloaded. A progressive decrease in the size of the soleus muscle from suspended rats was observed as a function of time. The rate of protein degradation of the homogenates from the soleus muscles of suspended and control animals was not significantly different. The rate of cell-free protein synthesis was severely repressed in the atrophied muscle. An initial rise in the levels of plasma glucose and corticosterone was observed on the second day of suspension, but they subsequently returned to normal values.

INTRODUCTION

The Soviet space experiments have demonstrated that exposure of rats to weightlessness invariably leads to atrophy of some skeletal muscles (1,2). The most seriously affected is the antigravity soleus muscle (2). At present, virtually nothing is known on the kinetics and biochemical mechanisms of muscle wasting that occurs during space flight. This study was undertaken to determine whether a hypokinetic rat model (3) can provide some definitive information on the mechanism of muscle degeneration in weightlessness.

MATERIALS AND METHODS

The experiments were conducted on male Munich-Wistar rats weighing between 150-200 grams. For each experiment, 3-4 rats were suspended in the hypokinetic model. A corresponding number of control rats were housed in the same environment. The description and use of this model has been reported elsewhere (3). Briefly, the rats on this model were suspended from a back-harness with the hind limbs fully unloaded and the head tilted downward. After various exposure periods, the animals were sacrificed by decapitation and the muscles were excised and weighed. One soleus muscle was used for protein degradation studies and the contra lateral muscle for cell-free protein synthesis. For proteolytic activity, the soleus muscle was minced, homogenized in Chappel-Perry buffer, at 4C, Ph 7.4, and centrifuged at 800xG for 30 minutes. To the supernatant containing between 3-5 mg of protein/ml, cycloheximide was added to a final concentration of $5x10^{4}M$ to prevent protein synthesis from taking place. The mixture was incubated at 37C for one hour and the rate of protein breakdown was determined fluorometrically (4) by measuring the amount of tyrosine present before and after incubation. For the cell-free protein synthesis measurement, soleus muscles from 3-4 rats were pooled, minced, homogenized, centrifuged at 30,000xG for 30 minutes and the postmitochondrial supernatant was tested for protein synthetic activity according to the method of Leon, et al. (5). The amount of $^{14}C(U)$-Leucine incorporated into muscle protein was used as an index of protein synthesis. Plasma corticosterone was done fluorometrically (6) and protein was determined as described by Bradford (7).

RESULTS AND DISCUSSION

The soleus muscle of rats suspended in the hypokinetic model exhibited rapid loss of muscle mass as a function of time (Table 1). Three days after suspension, there was a decrease of 7% in the size of this muscle, based on tissue wt./body wt., compared to control rats. After 5 days, a significant difference of 20% was observed. The size of the muscle decreased to a minimal level sometime between the 5th and 7th day. This minimal size probably represents a new steady state; further exposure, up to 15 days, did not result in any additional change. Other skeletal muscles also showed some changes. The gastronemius and the plantaris decreased in size, but not to the extent found in the soleus. The alterations in the extensor digitorum longus and tibialis anterior were insignificant. Coincidently, the reduction in size of the various muscles was very similar to that observed in the rats of the Cosmos experiments (1,2).

Atrophy of skeletal muscle is sometimes related to the rate at which muscle protein is degraded. The difference in the proteolytic activity of homogenates from the soleus muscle of suspended and control rats was indistinguishable during the first week of exposure (Table 2). This is the period in which the rate of atrophy of this muscle is at its maximum. On the other hand, when cell-free protein synthesis rate was measured during the same time interval, a significant time-dependent decrease in synthesis was observed in the muscle sample from suspended rats (Table 3). There was only a small change in the rate of synthesis at Day 3, but a severe repression of approximately 50% occurred at Day 5. The rate of protein synthesis was still considerably below control at Day 7.

The coincidence of finding of muscle atrophy, high levels of corticosterone and glucose, and hypertrophy of adrenal gland in rats exposed to weightlessness (8,9) raises an important question. Is glucocorticoid an important factor in the muscle wasting process that is induced by weightlessness? The rats placed on the hypokinetic model showed a temporary, but significant, increase in plasma glucose and corticosterone, and hypertrophy of the adrenal tissue on the second day of suspension. However, when subsequent measurements on glucose and corticosterone was made on Days 3, 5, and 7, they were similar to control. The adrenal gland, measured again on the 7th day, showed a regression to normal size.

The finding that there was no increase in the rate of protein degradation in muscle undergoing atrophy in the hypokinetic model is perplexing. However, if the data is valid, the net decrease in muscle size would have to be accounted for by a corresponding decrease in the rate of protein synthesis of the unloaded muscle. The results reported here support this prediction, but since it is an in vitro measurement, it should be interpreted with some caution. In contrast to this result, Musacchia and collaborators (10), using a slightly modified version of this model, found that the rate of muscle breakdown, determined by the urinary excretion rate of 3-methyl-histidine was significantly higher in the suspended animals compared to control. The reason for the discrepancy is unknown, however, it is possible that they were measuring the collective breakdown rate of other muscles, but, not the soleus. It has been reported that soleus muscle is devoid of 3-methyl-histidine (11).

Table 1. EFFECT OF SIMULATED WEIGHTLESSNESS ON THE SIZE OF RAT SOLEUS MUSCLE

Suspension (Days)		Tissue wt/body wt (mg/gm)	P
3	control	.359±.010	
	suspended	.333±.011	ns
5	control	.364±.007	
	suspended	.294±.007	< .001
7	control	.414±.009	
	suspended	.226±.013	< .001
10	control	.343±.012	
	suspended	.193±.009	< .001
15	control	.349±.006	
	suspended	.194±.014	< .001

Table 2. PROTEIN BREAKDOWN RATE OF HOMOGENATE OF ATROPHIED AND CONTROL MUSCLES

nM Tyrosine releaded/mg protein/hr

Exposure (Days)	Suspended	Control
3	1.21±.17	1.22±.20
5	1.27±.24	1.86±.23
7	1.10±.15	1.28±.24

Table 3. RATE OF CELL-FREE PROTEIN SYNTHESIS OF ATROPHIED AND CONTROL SOLEUS MUSCLES

Incorporation of L-^{14}C(U) Leucine into muscle protein

Exposure (Days)	cpm/mg protein/hr Suspended	Control	% Difference
3	727	811	10
5	442	902	51
7	797	1202	33

REFERENCES

1. Il'ina-Kakueva, Ye.I. and Portugalov, V.V. Arkhiv anatomii, gistologii i embrologii 76: 22-27, 1979. Translation, NASA TM-75482.

2. Kazaryan, V.A., E.A. Rapoport, L.A. Goncharova, and S.Ya. Bulycheva. Space Biol. and Aerospace Med. 11:23-27, N-6, 1977.

3. Morey, E.R. Bioscience 29:68-172, 1979.

4. Waalkes, T.P. and S. Udenfriend. J. Lab. Clin. Med. 50:733-736, 1957.

5. Leon, H.A., D.D. Feller, E.D. Neville, and B. Daligcon. Life Sciences 4:737-741, 1965.

6. Scriba, P.C. and O.A. Muller. In: Methods of Hormone Analysis (H. Breuer, E. Hamel, and H. Krupkemper (eds) pp. 203-210, John Wiley and Sons, New York, 1975.

7. Bradford, M.M. Anal. Biochem. 72:248-254, 1976.

8. Gazenko, O.G., A.G. Genin, Ye. A. Il'in, V.V. Portugalov, L.V. Serova, and R.A. Tigranyan. Space Biol. and Aerospace Med. 12:54-63, N-6, 1978.

9. Kvetnyanski, R., R.A. Tigranyan, T. Torda, and D. Repchekova. Space Biol. and Aerospace Med. 14:30-35, N-6, 1980.

10. Musacchia, X.J., D.R. Devers, G. Meininger, and T.P. Davis. J. Appl. Physiol. 48:479-486, 1980.

11. Kuehl, W.M. and R.S. Adelstein. Biochem. Biophys. Res. Comm. 39:56-964, 1970.

THE EFFECT OF HYPOKINESIS AND HYPOXIA ON THE FUNCTION OF MUSCLES

T. Szilágyi, J. Hideg[+], A. Szöőr[++], E. Berényi[+], M. Rapcsák, and A. Pozsgai[+]

Institute of Pathophysiology and Physiology[++], Medical University, 4012 Debrecen. Pf. 23; Hungarian People's Army Medical Corps. [+]

It was given a report by Hideg et al. (3) on the influence of hypokinesis on functionally different skeletal muscles. In present experiments we examined the effect of hypokinesis, hypoxia and the effect of the two methods applied together on rats.

METHODS. Adult male rats belonging to CFY strain were used. The animals of the first group were put in a special small plastic cage in which their motion was restricted. Water and food was given "ad libitum" with the aid of selffeeders applied to the head part of cages. The other group of the animals was exposed to intermittant hypoxic state. We kept the rats in hypoxia (corresponding to 7000 m altitude above sea level) for 8 hours/day. In the third group we kept the animals under hypokinetic conditions and they were exposed to intermittant hypoxic load as well. We had a control group of rats living under normal conditions in the laboratory.

2, 4 and 8 weeks after we started our experiment, we killed the rats by exsanguination through aorta abdominalis. The following muscles were excised for experiments: extensor digitorum longus (EDL), gastrocnemius and soleus.

The total lipid content of the muscles was investigated by the method of Cristopher et al. (1). The lipid fractions were separated after being extracted according to Folch et al. (2) on thin layer plates of silicagel. - The contractile properties of muscles were studied by the method of Szöőr et al. (4).

RESULTS. The body weight of the rats kept in the cage increased more intensely than that of the other group kept in hypoxia during the first 4 weeks, in the second half of the given period the body weight of hypoxic animals was greater than that of hypokinetic ones. The body weight of animals exposed to combined treatment was stagnant during the first 2 weeks, it increased in the following 2 weeks, then it decreased so they gained about 20 g altogether during the 8 weeks (Fig. 1.).

Fig. 2. shows the weight variations of EDL. After 2 weeks the hypokinetic group showed only a slight difference compared to the control one, a significant difference between them could only be detected during the fourth week. In the case of hypoxia and hypoxia combined with hypokinesis we saw a significant decrease in weight after 2 weeks, in the latter group the weight of EDL decrease by 50% on the 8th week. The weight of gastrocnemius muscle showed a significant decrease during the second week in all the groups. The greatest decrease occured in the combined group, which on the 8th week went up to 40%. The weight of soleus muscle of hypokinetic rats decreased on the 8th week only. It is interesting that the weight of soleus muscle of the animals exposed to combined treatment was less atrophied than that of those kept in hypoxia. - We also examined the dry weight of the muscles of the rats exposed to different treatment, but no significant difference was observed.

We investigated the variations in the total lipid content of the muscles; in the case of EDL that increased in the hypokinetic and hypoxic groups, whereas in the case of the animals exposed to combined treatment it was equivalent to that of control ones. After 8 weeks the most significant increase could be seen in the hypoxic group. The total lipid content of m. gastrocnemius decreased significantly in all the groups after 2-4 weeks, but on the 8th week it was equal to that of control group. In m. soleus the lipid content increased significantly only in the group exposed to combined treatment after 2 weeks, on the 4th week the increase went up to 50% of the control in the group exposed to hypoxic and combined treatment. However, the 8th week all the parameters approached the control ones.

According to chromatogram examination a significant increase or decrease occured in some lipid fractions on the 4th week but these parameters approached the control values on the 8th week. As it can be seen (Fig. 3) free fatty acid, triglyceride, cephalin and lecithin fractions accumulate in EDL. In m. gastrocnemius cholesterol ester disappears

while cephalin and lecithin decrease. In m. soleus we can see the accumulation of cholesterol ester, cholesterol, cephalin and lecithin.

Furthermore we examined the contractile properties of muscles. If we correlated the muscular strength with one unit of circumference of the muscle fiber we could see a decrease of small degree. On the other hand when we correlated our results with the whole mass of the muscle a significant decrease could be observed in the case of EDL.

To sum up our results we can say (5) that plaster cast immobilisation causes faster developing and more pronounced structural and functional changes in all three types of muscles - first of all in antigravitational soleus muscle. These changes are similar to those observed in weightlessness. Hypokinesis causes slight changes which develop slowly so it is more difficult to analyse them. It can be said that hypoxia intensifies the effect of hypokinesis in general. The decrease in muscular strength must be due to atrophy and becoming adipous at the same time.

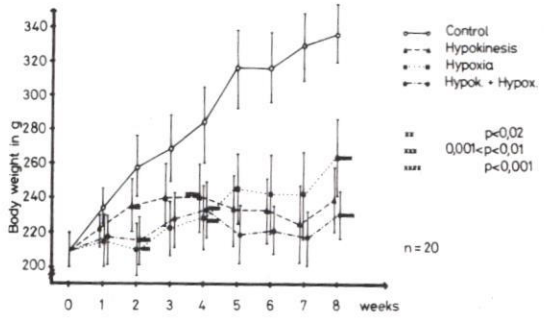


Fig. 1. Body weight variations of rats

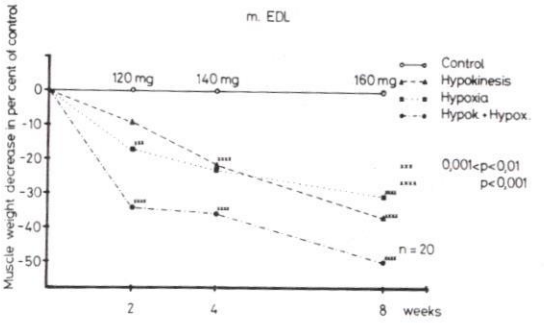


Fig. 2. Weight variations of EDL

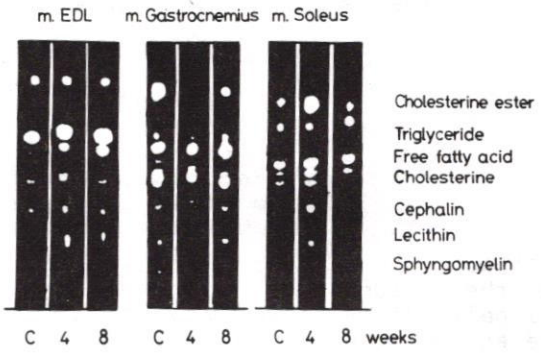


Fig. 3. Chromatogram of lipid fractions (hypokinesis + hypoxia)

REFERENCES

1. Cristopher, S., T. Frings, W. Frendley, R.T. Dunn, C.A. Aqueen. 1972. Improved determination of total serum lipids by the sulphophosphovanillin reaction. Clin. Chem. 18: 673-674.
2. Folch, S., M. Lees, G.H. Sloane-Stanley. 1957. Simple method for the isolation and purification of total lipids from animal tissues. J. Mol. Biol. 226: 497-501.
3. Hideg, J., Ö. Takács, É. Berényi, M. Rapcsák, F. Guba. 1981. Influence of hypokinesis on functionally different skeletal muscles of rats. In: Adv. in Physiol. Sci. Vol. 19. Gravitational Physiology pp. 153-161. Pergamon Press. Akad. Kiadó.
4. Szöőr, Á., M. Rapcsák, A. Boross. 1981. Short procedure for preparation of glycerol treated muscle fibres. Acta Biochim. Biophys. Acad. Sci. hung. (in press).
5. Szilágyi, T., Á. Szöőr, Ö. Takács, M. Rapcsák, V.S. Oganov, S.A. Skuratova, S.S. Oganesyan, L.M. Murashko, M.A. Eloyan. 1980. Study of contractile properties and composition of myofibrillar proteins of skeletal muscles in the Cosmos-1129 experiment. The Physiologist. 23: S-67-70.

ANALYSIS OF TRANSIENT CARDIOVASCULAR RESPONSE TO ORTHOSTATIC STRESS USING NONINVASIVE METHODS

H. Pessenhofer, G. Schwaberger, N. Sauseng, T. Kenner

Department of Physiology, Karl-Franzens-University, Graz, Austria

ABSTRACT

The application of noninvasive methods for the analysis of dynamic control phenomena in the cardiovascular system on clinical and space research problems is demonstrated. In an investigation, performed in 10 volunteers, the transient responses of heart period, systolic time intervals and impedance cardiogram were recorded immediately after a sudden change in body position from horizontal to vertical (tilt-table), beat-per-beat for one minute. To estimate the time course of variables which could not be directly determined by noninvasive methods, we additionally used a mathematical model of the physiological system, the free parameter of which were estimated by means of a systems identification procedure. The resulting time functions of the measured variables and of the variables calculated on the basis of the model (mean arterial pressure, enddiastolic filling pressure, total peripheral resistance) demonstrated the interplay between baroreceptor reflex and Frank-Starling-mechanism in the adjustment of the mean arterial pressure after a change in body position.

INTRODUCTION

Current test procedures for the assessment of orthostatic tolerance for clinical and space research purposes [2,6] take the steady state values of some diagnostic variables in response to a certain test signal (e. g. change in body position or lower body negative pressure) as measures for orthostatic stability.
Because the cardiovascular control system is a dynamic feedback control system it should be stressed, that only the knowledge of both magnitude and time course of cardiovascular response to a test signal allows a characterization of the cardiovascular control system in total.
The application of noninvasive methods for the determination of various cardiovascular variables in the course of an investigation of cardiovascular control seems essential, because these variables are very sensitive to fluctuations, e. g. caused by the sensation of pain.
In the following we try to demonstrate the transient response of cardiovascular variables, obtained by noninvasive methods, in the early phase of orthostatic response using a sudden change in body position, performed via a tilt-table, as test signal.

METHODS

10 healthy volunteers (age of 18 years in average) were subjected to a test protocol consisting of three phases: a) control phase-horizontal (lasting 15 minutes), b) transient phase (sudden and unexpected change of tilt-table position to vertical by means of a pneumatic control system, lasting 1 minute), c) control phase-vertical (lasting 15 minutes).
The recorded primary variables were: ECG, phonocardiogram, sphygmogram of the carotid artery, impedance cardiogram (resp. first derivative of impedance cardiogram) [4] and blood pressure by an automatic cuff device during the control phases.
The secondary variables, derived from the primary variables were: heart period, systolic time intervals [5] (duration of electromechanical systole, left ventricular ejection time and preejection period) and relative changes of stroke volume.
All secondary variables were determined every minute during the control phases and beat-to-beat during the transient phase.
To estimate the transient response of mean arterial pressure, total peripheral resistance and enddiastolic filling pressure, a mathematical model of the left ventricle and the arterial system [3, 7], not to be discussed here in detail, was used.
The free parameter of the model were estimated on the basis of the directly recorded secondary variables using a systems identification procedure [1].
That way the calculation of variables, not to be determined in a straight forward manner by noninvasive methods was made possible.

RESULTS

From the individual time functions of the secondary variables we calculated an average response of all volunteers as mean value of the individual responses at equidistant intervals of time. Figure 1 demonstrates the mean transient responses of heart period and systolic time intervals, the moment of tilting is represented by the origin of the abszissa.
Figure 2 depicts the estimated transient responses of mean arterial pressure, total peripheral resistance and enddiastolic filling pressure, calculated from the model.

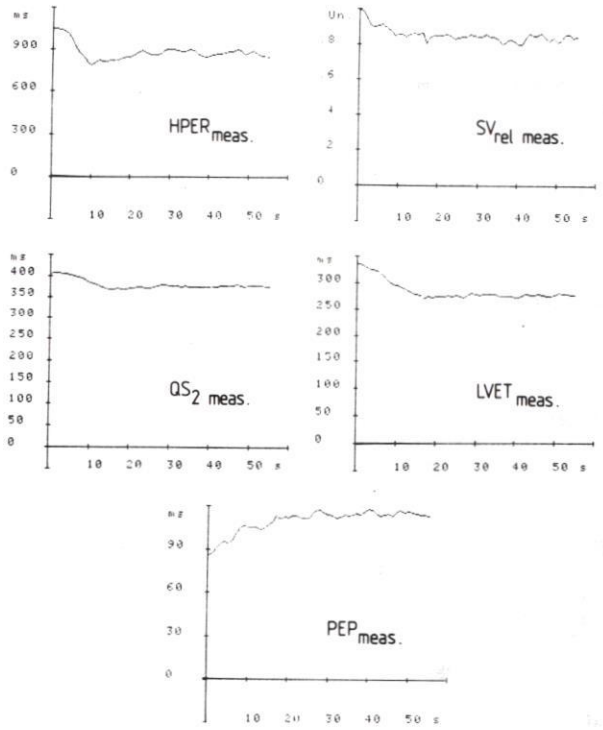


Figure 1: Measured transient responses of heart period (HPER), relative stroke volume (SV), duration of the electromechanical systole (QS_2), left ventricular ejection time (LVET) and preejection period (PEP).

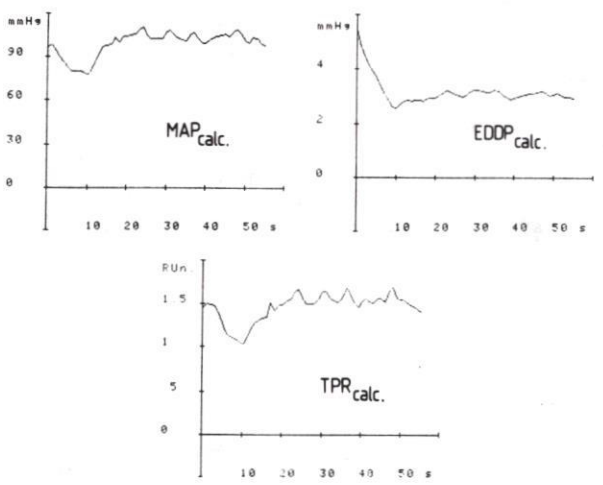


Figure 2: Calculated transient response of mean arterial pressure (MAP), left ventricular end diastolic filling pressure (EDDP) and total peripheral resistance (TPR).

DISCUSSION

The transient responses of different cardiovascular variables (figure 1 and 2) demonstrate the dynamic process of cardiovascular adaption to altered body position. Concerning the reliability of the estimated time functions of mean arterial pressure, total peripheral resistance and end diastolic filling pressure, calculated on the basis of the model, it should be mentioned, that the absolute values are - to some extent - subjected to interpersonal errors because of the more statistical nature of the model assumptions. The responses, however, correspond well with data presented in the literature [6].

The whole set of response curves opens the possibility to a more detailed differentiation between action of the preload (Frank-Starling-mechanism), represented by the end diastolic filling pressure, and the baroreceptor reflex action in response to orthostatic stress.

An appropriate quantification, as in general use in control theory, provides means for clinical and statistical evaluation of the responses also in the case of disturbancies of orthostatic tolerance as to be seen, for example, after prolonged adaption to weightlessness.

We therefore think that an investigation of the dynamic transient response of the cardiovascular system to orthostatic stress, provoked either by tilting or by lower body negative pressure tests, should be a fixed part of preflight and postflight test of crewmen in manned spaceflight.

REFERENCES

[1] EYKHOFF, P.: System-Identification - Parameter and State Estimation, J. Wiley & Sons New York, London, Sidney 1974

[2] JOHNSON, R.L., HOFFLER,G.W., NICOGOSSIAN, A. E., BERGMAN, S.A., JACKSON, M.M.: Lower Body Negative Pressure: Third Manned Skylab Mission, in: Biomedical Results from Skylab, ed.: R.S. JOHNSON, L.F. DIETLEIN, NASA, Washington D.C. 1977

[3] KENNER, T.: Physical and Mathematical Modeling in Cardiovascular Systems, in: Quantitative Cardiovascular Studies, ed.: N. C.H. HWANG, D.R. GROSS, D.J. PATEL, Univ. Park Press, Baltimore 1979

[4] KUBICEK, W.G., KOTTKE, F.J., RAMOS, M.U., PATTERSON, R.P., WITSOE, D.A., LABREE, J. REMOLE,W., LAYMAN, T.E., SCHOENING, H., CARAMELA, J.T.: The Minnesota Impedance-Cardiograph - Theory and Applications Biomed. Eng. 9, 410-416 (1974)

[5] LEWIS, R.P., LEIGHTON, R.F., FORRESTER, W.F. WEISSLER, A.M.: Systolic Time Intervals in: Noninvasive Cardiology, ed.:A. M. WEISSLER, Grune & Stratton, New York 1974

[6] MAREES, H. de.: Zur orthostatischen Sofortregulation, in: Neural Control of the Cardiovascular System and Orthostatic Regulation, ed.: H. DENOLIN, J.C. DEMANET, S. Karger, Basel, München, Paris 1976

[7] SUGA, H., SAGAWA, K.: Instantaneous Pressure Volume Relationships and their Ratio in the Excised and Supported Canine Left Ventricle
Circ. Res. 35, 117-126 (1974)

ANTIORTHOSTATIC HYPOKINESIA AND CIRCULATION IN THE RAT

Vojin Popovic

Department of Physiology, Emory University
School of Medicine, Atlanta, GA. 30322

ABSTRACT

Using an animal model that was developed in our laboratory, we have studied circulatory mechanisms that occur during exposure to head-down hypokinesia as well as during readaptation of animals to control conditions. Unanesthetized, unrestrained Sprague-Dawley rats (200 ± 10g) with chronically cannulated aorta and right ventricle of the heart are used in the experiments. This is done because surgery and anesthesia drastically decrease cardiac output. Seven days long exposure to hypokinesia induced the following changes: The heart rate was slightly elevated. Right atrial pressure increased to 4 mm Hg, but returned to 0 mm Hg after 3 days of exposure. Mean arterial blood pressure decreased from 118 to 100 mm Hg during early exposure to antiorthostatic hypokinesia. The return to the normal value was observed after two days of hypokinetic exposure. Cardiac output and stroke volume of resting rats were increased during early exposure to hypokinesia, but decreased continuously during the next seven days. The results indicate that antiorthostatic hypokinesia induces similar circulatory changes seen in astronauts after several days of exposure to 0 g forces.

INTRODUCTION

Gravity has a profound effect on mammalian organisms. The effect of gravity on the cardiovascular system is especially great. It has been reported that during exposure of men to weightlessness certain circulatory adaptations occur. After return to earth new circulatory readaptations have been observed. The changes induced by weightlessness include shifts in blood distribution, overloading of atria, neurohumoral stimulation leading to excessive water loss and a profound blood volume decrease concomitant with other circulatory changes. These circulatory changes lead to orthostatic intolerance and a decreased work ability after return to earth.

Hypokinesia, especially antiorthostatic hypokinesia, mimics the effects of weightlessness. This has been shown in animal experiments for bone turnover (1), nitrogen and cation metabolism (2) and in human experiments. Hypokinesia mimics in a high degree the effects of 0 g conditions on the cardiovascular system. Using a rat model that was developed in our laboratory we are presently studying circulatory mechanisms that occur during exposure to hypokinesia. The cardiovascular measurements are performed in control experiments on unrestrained, unanesthetized rats and in the same animals in hypokinetic conditions or during readaptation to free activity. It is hoped that the study will ultimately serve to predict and to identify possible circulatory mechanisms in animals exposed to Space Shuttle/Space Lab conditions.

METHODS

Unanesthetized, unrestrained, resting Sprague-Dawley rats (200 ± 10 g) were used in the experiments because surgery and anesthesia drastically decrease cardiac output and other circulatory parameters (3). Aorta and right ventricle of the animals were permanently cannulated fifteen to twenty-one days before the experiment. Heart rate, arterial and central venous blood pressure and cardiac output were measured before, during seven day long antiorthostatic hypokinesia and during recovery from hypokinesia. Holton-Musacchia system (1,2) for inducement of hypokinesia was used. The system includes 1) the ability for the animal to exercise using only his front limbs, 2) a fluid shift and 3) total unloading of the rear limbs. The head of the animals is tilted down 20°.

RESULTS

The resting mean arterial blood pressure was decreased during initial 48 hours of exposure to hypokinesia, while the heart rate of the rats was slightly increased. From the third day on the arterial blood pressure and the heart rate returned to the normal values. The right atrial pressure rose the first day of hypokinesia from 0 to +5 mm Hg. The second day the right atrial pressure declined and from the third day of hypokinesia it was at the same level as in control prehypokinetic conditions. The cardiac output and stroke volume were increased after exposure to hypokinesia. They decreased during the next three to four days until it became lower than in control animals. During readaptation to normal activity, heart rate was elevated during a period of 7-14 days while cardiac output returned to the normal prehypokinetic values after 3 days. Mean arterial blood pressure stayed unchanged.

In conclusion, our results indicate that antiorthostatic hypokinesia induces similar changes in animals that are observed in man after exposure to weightlessness. The rats with chronic aortic and right atrial cannulas seem to be the animals of choice because the cannulas stay patent for the whole life span (4).

REFERENCES

1). Morey, E.R., E.E. Sabelman, R.T. Turner and D.J. Baylink. A new rat model simulating some aspects of space flight. Physiologist 22. S23-S24, 1979

2). Musacchia, X.J., D.R. Deavers, G.A. Meininger and T.P. Davis. A model for hypokinesia: effects on muscle atrophy in the rat. J. Appl. Physiol.: Respirat. Environ. Physiol. 48: 479-486, 1980.

3). Popovic, V., and K.M. Kent. 120-day study of cardiac output in unanesthetized rats: Am. J. Physiol. 207, 767-770, 1964.

4). Popovic, Pava, V. Popovic, R. Schaffer, and A.S. McKinney. Effect of levodopa on arterial blood pressure in unanesthetized and in anesthesized rats. Proc. Soc. Exp. Biol. Med. 154, 391-396, 1977.

This work was supported by NASA grant NGR-11-001-009.

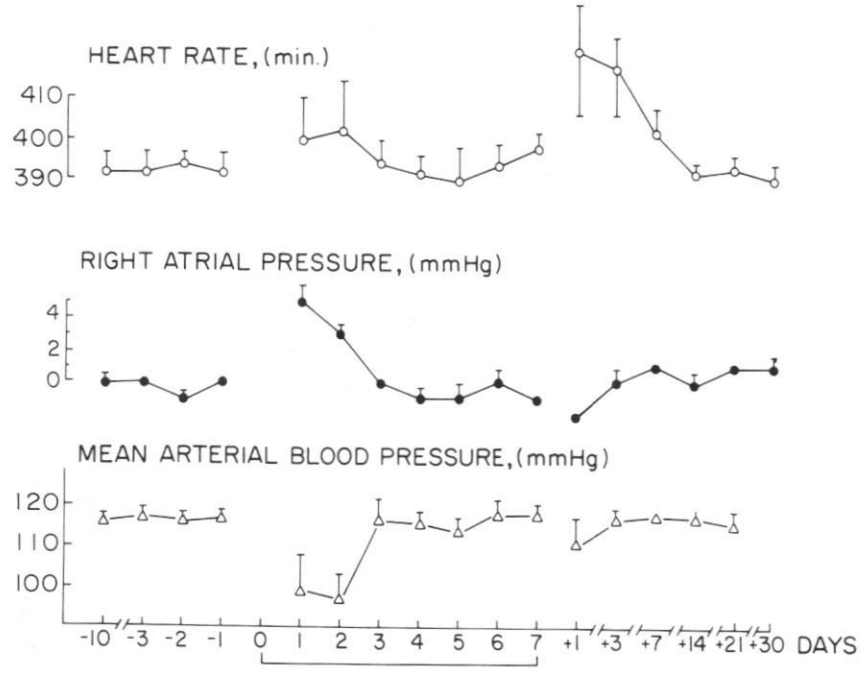


Figure 1. Heart rate, right atrial pressure and mean arterial blood pressure (±SD) of eight rats prior, during and after exposure to hypokinesia with -30° tilt.

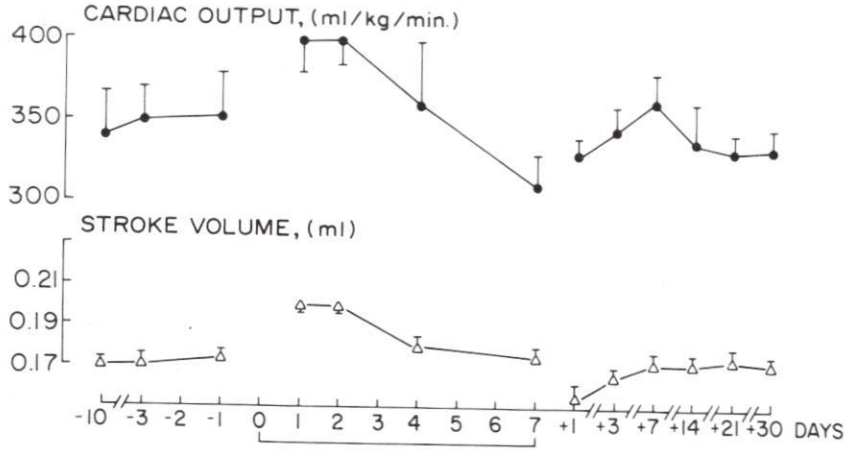


Figure 2. Cardiac output and stroke volume during and after seven days long hypokinesia.

PROTEIN COMPOSITION OF mRNA CONTAINING 18S-40S SUBRIBOSOMAL PARTICLES IN SOLEUS MUSCLE BEFORE AND AFTER IMMOBILIZATION

I. Sziklai, J. Székely, Zs. Kiss, F. Guba

Department of Oto-rhino-laryngology, P.O.B. 422, Dept. of Neurology, Inst. Biochemistry, Univ. Med. School, Szeged H-6701, Hungary

INTRODUCTION

Due to weightlessness and hindlimb immobilization, the type specific slow isomyosins of the antigravitational soleus muscle are substituted in part by fast isomyosins. There has so far been no explanation of this phenomenon. Reports have recently been published about the in vitro translation of non-polysomal mRNA occuring freely in the sarcoplasm and responsible for actin synthesis /1-2/. We assume that these free mRNA-protein complexes also contain the codon of the fast isomyosins, but not in translatable form, because of the masking proteins in the soleus muscle. Immobilization may produce a sign making possible translation for these mRNA copies as well.

MATERIALS and METHODS

Subribosomal particles containing mRNA protein complexes /mRNP/ were isolated from normal and immobilized /for 4 wks/ soleus muscles of rabbit, as described by Bag and Sarkar /1/. The 18S-40S subribosomal particles /RNP/ containing mRNP were analysed on a 5-20% linear sucrose density gradient, with centrifugation at 117.000x g for 16 hrs in a Beckman SW 27.1 rotor. The protein compositions of the 18S-40S RNP were examined by two-dimensional gel electrophoresis / 2-D PAGE/: isoelectric focusing in the first dimension /3/, and 10% sodium dodecyl sulphate polyacrylamid gel electrophoresis / SDS PAGE/ in the second. The trichloracetic acid /TCA/-soluble proteins were extracted from the sarcoplasm and nucleoplasm by the method of Seyedin et al./4/. The nonhistone proteins were extracted as described earlier / 5a,b/.

RESULTS and DISCUSSION

We have examined the subribosomal particles sedimenting between 18S-40S during 5-20% linear sucrose gradient ultracentrifugation /Fig. 1/. The protein composition of these particles showed heterogeneity between 30-99 kD./Fig. 2.a/ Isoelectric focusing of the proteins of the 18S-40S RNP isolated from normal and immobilized soleus muscles revealed qualitative differences. During immobilization the pH 5.5 fraction disappeared from the pattern, while the basic components /pH 7.5 and 7.6/ remained unchanged./Fig. 2.b/

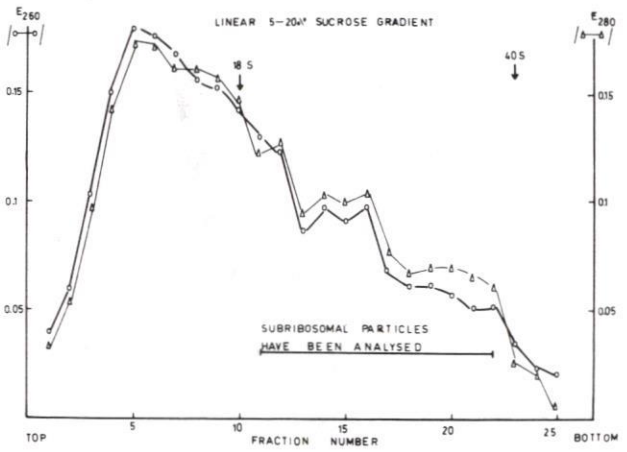


Fig. 1. Sedimentation of subribosomal particles on a 5-20% sucrose gradient

Co-focusing with 3-20% TCA-soluble nuclear proteins demonstrated identical isoelectric points between the basic fractions of the 18S-40S proteins and the minor components of TCA-soluble nuclear proteins/Fig. 2.a, b/. There were, however, no identical spots with major TCA-soluble sarcoplasmic proteins. The protein fraction disappearing during immobilization was separated into several spots, having pH between 4.5 and 5.8 and molecular weight /mol.weight/ between 35 and 38.5 kD. by 2-D PAGE /Fig. 3.b/ These differences were also detectable by SDS PAGE / Fig. 2.a/. The similarity of the 18S-40S RNP proteins with the TCA-soluble nuclear proteins could also be seen. The proteins disappearing from the 18S-40S RNP during immobilization were not identical with any of the nuclear nonhistone proteins /Fig. 3.c/.

The protein composition of the 18S-40S RNP changed qualitatively during immobilization in the soleus muscle. Some polypeptides eliminated from the complexes were acidic in character, with mol. weight 35-38.5 kD. These proteins were not identical with nuclear nonhistone proteins, and were

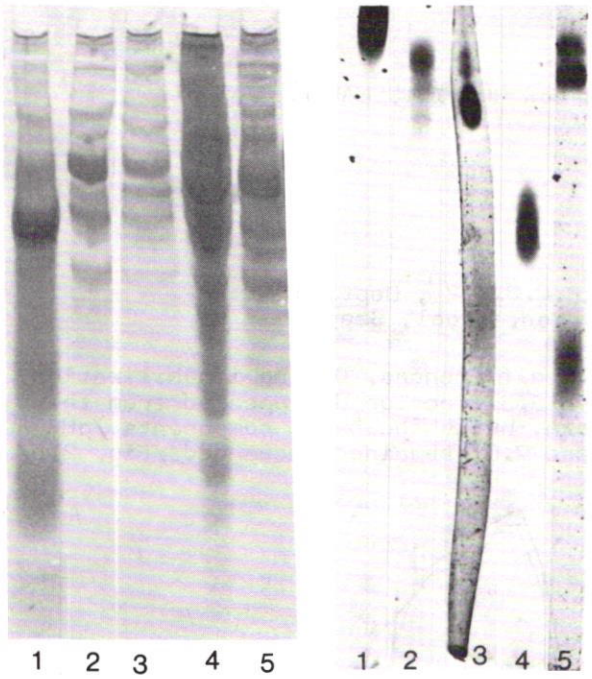


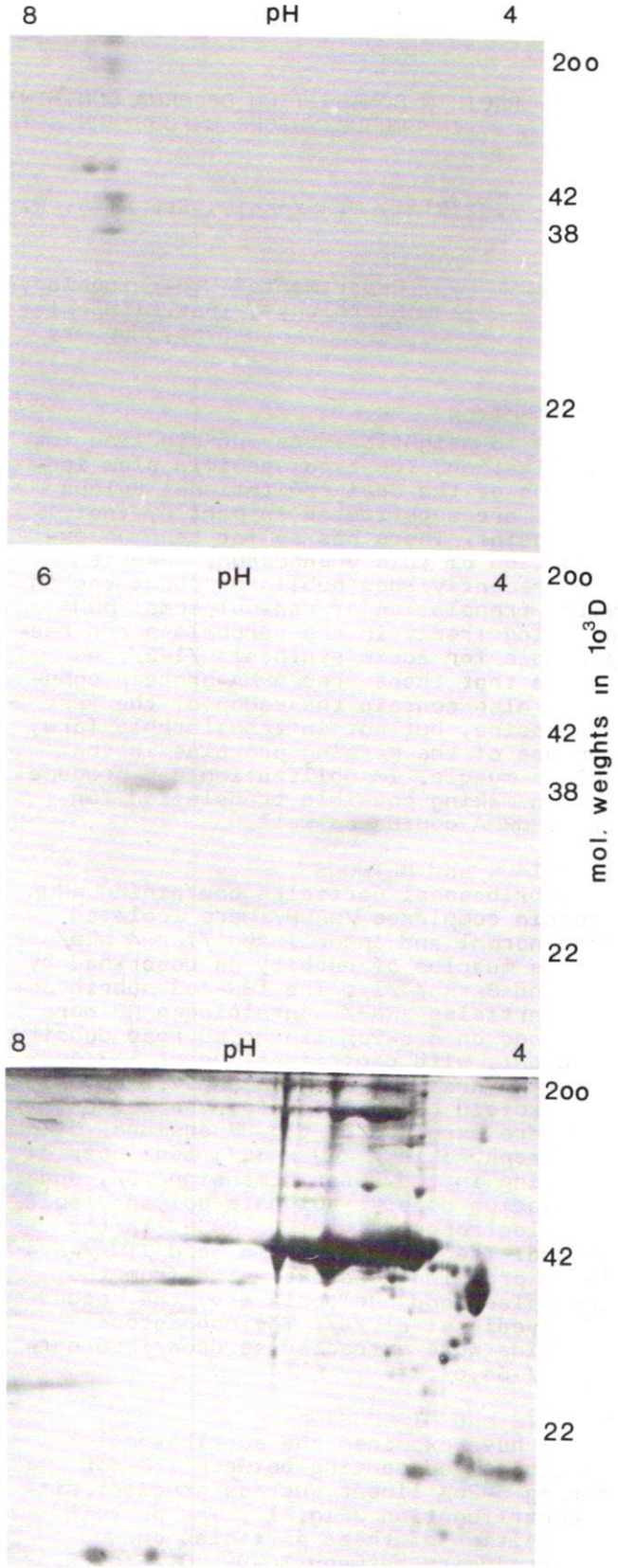
Fig.2.
a, 1o% SDS PAGE of 18S-4oS RNP fractions of normal /NS/ and immobilized /IS/ soleus muscle
b, Isoelectric focusing of 18S-4oS RNP proteins
1. TCA-soluble sarcoplasmic proteins /NS/
2. TCA-soluble nuclear proteins /NS/
3. 18S-4oS RNP proteins /IS/
4. Polysomal proteins /NS/
5. 18S-4oS RNP proteins /NS/

Fig.3. Two-dimensional PAGE of
a, 18S-4oS RNP from immobilized soleus muscle /4o ug protain loading/
b, Proteins disappearing from 18S-4oS RNP pattern during immobilization/carrier Ampholine:pH 4-6
c, Nonhistone proteins from normal soleus muscle with large-scale microseparative technique

---

not derived from ribosomal subunits, as there were no other lower molecular weight proteins similar to the polysomal ones. The change of the sarcoplasmic, free 18S-4oS RNPs /containing mRNP/ may be closely connected with the transformation of myosin isoenzyme synthesis during weightlessness and immobilization. In our view the mRNA content of the analysed RNP fractions can be the copy /among some others/ for the fast isomyosin synthesis in the slow muscle, persisting in the sarcoplasm in a non-translatable form during normal life.

References (see page 54).



EFFECTS OF PROLONGED BEDREST IN ANTIORTHOSTATIC POSITION ON rCBF MEASURED BY 133Xe INHALATION TECHNIQUE : EFFECTS OF CLONIDINE.

A. GUELL, G. VICTOR, A. BRU, P. MONTASTRUC, A. BES

Department of Neurology, CHU TOULOUSE RANGUEIL 31054 TOULOUSE (France)

Experiments of prolonged bedrest in antiorthostatic position are conducted in order to simulate cardio-circulatory modifications observed in weightlessness. Until now, no studies of rCBF have been effected in these conditions.
6 young, healthy volunteers were placed in strict bedrest and in antiorthostatic position -4° for 7 days. The rCBF measurements were studied by 133Xe inhalation method using a 32 detectors system. Studies were made in basal conditions, then between the 6th and the 12th hour, and finally between the 72 and 78th hour after the beginning of the experiment. 3 of the subjects received 0,450 mg of Clonidine daily during the experiment. In the subjects having taken no Clonidine, we observed a constant increase in rCBF in the first 12 hours ; at the 72nd hour, all values hade returned to basal stade. These findings agree with a well known notion of a rapide correction of hemodynamic disturbances observed in the first days of weightlessness.
In the subjects treated with Clonidine, the increase of rCBF did not occur. Several mechanisms of action are possible ; the Clonidine affecting either the heart, or the brain by direct vaso-constriction.

Space flights cause an important redistribution of the circulating blood mass in the human cardio-vascular system. The redistribution goes from the infracardiac regions towards the cephalic region (3). On earth, it is impossible to simulate weightlessness ; there are however a few conditions such as immersion (6), prolonged bedrest (8) and antiorthostatism (7) that allow us to recreate, in a much weaker way, some of the changes that occur in space, and those, incidentally, that concern the cardio-vascular system. In this work, we studied the possible cephalic circulatory changes by means of following the variations of regional Cerebral Blood Flow (rCBF) during simulated 0 gravity.

MATERIAL AND METHOD

6 young healthy volunteers (median age : 24 years + 4, 1 years) were placed in bedrest and in a -4° antiorthostatic (head down) position for 7 days ; they were not allowed to raise their head from the level of the bed but they were still allowed to move lateraly. 3 of these subjects received, for the entire length experiment, Clonidine, 0,450 mg per day in three doses for to block the gauer's reflex (5).
To measure the possible variations of the rCBF we used a multidetector apparatus that was equiped with 16 scintillation detectors for each hemisphere which was based on the Xenon 133 inhalation technique that OBRIST and RISBERG have perfected (10, 11). 3 rCBF were performed on each of the volunteers : the first one, before bedrest began and before receiving Clonidine; the second one, between the 6th and the 12th hour after the beginning of the experiment ; finally, the third one, 72 hours after bedrest began.

RESULTS

Among the 3subjects who did not receive Clonidine, there was a constant increase in the rCBF (Fl : flow in grey matter) between the 6th and the 9th hour after the begining of the experiment : +13 %, +16 %, +17% respectively (figure 1). 72 hours after bedrest began, the rCBF does not show any significant variation with the results obtained before the bedrest.
Among the 3 subjects who received 0,450 mg Clonidine per day during the time of the experiment, we did not notice any significant variation in the rCBF; not even during the second measure.(Figure 1)
Finally, we noted that the subjects who did not receive Clonidine showed objective signs of cephalic "venous congestion" ; these symptoms appear 2 hours after the beginning og bedrest and reach their peak around the 48th hour. On the contrary, those who received Clonidine hardly presented any such symptoms during the experiment and when they appeared, they only did so towards the end of the experiment.

DISCUSSION AND CONCLUSION

It is well established that prolonged bedrest in antiorthostatic position causes a redistribution of the circulating blood mass in favor of the thoraco-cephalic region and most of all towards the head where a venous stasis will appear (4,7). This stasis will be responsable for a great variety of objective and subjective symptoms : feeling of head fullness, retinal venous dilatation, important facial oedema. The symptoms are present among the subjects that did not receive Clonidine and they are classically commond among all cosmonauts.
One can suppose that this venous stasis is responsable for a venous pressure increase in the intra cerebral veins; CO2 would then be less will eliminated and the cortical pCO2 would tend to increase; this in return could cause a vasodilatation in the arterial territory, thus, an increase in CBF,(1).

In favor of this hypothesis, one can recall the results of certain authors, found during animal experimentation, concerning the vasodilatation of pial arteries after an increase of venous pressure, (12).
When given, Clonidine will mask the increase in CBF that occurs in the other three patients by diminishing the cardiac output and by stimulated the local alpha2 adrenergic receptors.
It therefore appears that the experiments using prolonged bedrest in a -4° antiorthostatic position can be considered to be good experimental models for on the ground stimulation of the cephalic circulatory modifications that occur during space flights.
As a result of such research it should be possible to create a space pharmacopeia that could care for, and prevent the customarry problems that occur during the first part of the flight.

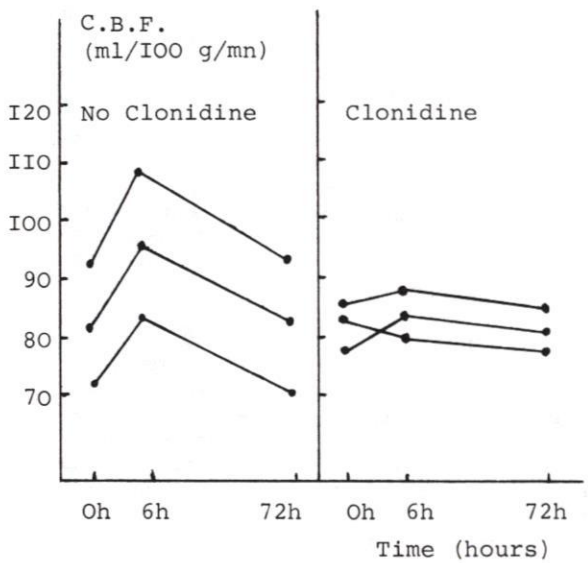


Figure 1 : effects of prolonged bedrest in a -4° antiorthostatic position on Cerebral Blood Flow : action of Clonidine.

REFERENCES

1. BES A. (1980) - Regulation de la Circulation Cérébrale. In : Journées d'Enseignement et d'Actualités Neurologiques. Ed. by CHIBRET, CLERMONT-FERRAND, pp 35-47.
2. BOLME P., FORSYTH R., ISHIZAKI T., MELMON L. (1975) : Hemodynamic effects of systemic and central administration of Clonidine in the Monkey. American Journal of Physiology, 222, 4, pp 1276-1276.
3. DIETLIN L.F. (1971) : Space flights deconditionning and overview of manned space flights results ?
N A S A, SP, 269, pp 1-26.
4. GUELL A., BRAAK L., ARNE-BES M.C., FANJAUD G. BES A. (1979) : Simulation d'absence de pesanteur: modifications circulatoires au niveau de l'extrémité céphalique. In : Circulation Cérébrale,Edit. A. BES & G..GERAUD, TOULOUSE, pp 271-274.
5. GUELL A., COLOMBANI J.C., GHARRIB Cl. MONTASTRUC P., BES A. (1981) : Effets de la Clonidine sur le métabolisme hydroélectrolytique chez des sujets placés en décubitus prolongé et en position anti-orthostatique.
XXIXth Inter. Congress Aviation and Space Medecine, 7-11 Septembre 1981, NANCY (France)
6. HOOD W.B., MURRAY R.H., URSHEL C.W., BOWERS J.A. and GOLDMAN J. (1948) : Effects of water immersion upon human subjects.
Aerospace Med., 39, pp 579-584.
7. KAKURIN L.I., LOBANIK V.I., MIKHAILOU M., SENKEVICH Y.A. (1976) : Anti-orthostatic hypokinesia as method of weightlessness simulation.
Aviation Space Environn. Med., 47, pp 1083-1086.
8. KOTOVSKAYA A.R., KAKURIN L.I., KONOVA N.I., SIMPURA S.F. and GRISHIWA I.S. (1965): Effects of prolonged hypokinesia on human resistance to G. Forces. In : Problems in Space Biology, Vol. 4 WASHINGTON, D.C., A.T.D., Press, pp 17-19.
9. MONTASTRUC P., MONTASTRUC J.L., DANG-TRAN L. : Clonidine inhibits negative pressure breating (or left atrial distension) induced diuresis : evidence for involvement of central alpha2 mechanisms (in press).
10. OBRIST W., THOMPSON H., KING C. (1967) : Determination of CBT by inhalation of 133 Xenon.
Circulation Research, 20, pp 124-135.
11. RISBERG J., ALI Z., WILSON E., WILLS E., HALSEY J. (1975) : Regional Cerebral Blood Flow by 133 Xenon Inhalation.
Stroke, 6, pp 142-148.
12. WET E.P. and KONTOS H.A. (1981) : Responses of pial arterioles to increased venous pressure.
Journal of CBF and Metabolism, Vol.1, Suppl. 1, pp 329-330.

SUSPENSION RESTRAINT: INDUCED HYPOKINESIA AND ANTIORTHOSTASIS AS A SIMULATION OF WEIGHTLESSNESS

X. J. Musacchia,* Joseph M. Steffen,* and D. R. Deavers**

*Dept. Physiol. & Biophys., Univ. Louisville, Louisville, KY 40292
**Dept. Physiol. & Pharmacol., Coll. Osteopathic Medicine & Surgery, Des Moines, IA 50213

ABSTRACT

Muscle, renal, fluid and electrolyte responses were measured in suspended rats; the hind limbs are non-load bearing and the front limbs can be used for feeding and grooming. Hind limb hypokinesia reverses after removal from the suspension harness. This suspension system is adjustable for a head-down tilt to produce antiorthostatic responses which are also reversable. Responses to hypokinesia or antiorthostatic hypokinesia for up to 14 days were measured; eg. muscle atrophy: soleus>gastrocnemius=plantaris>extensor digitorum longus, kaliuresis, and increased excretion of urea, NH_3 and 3 methylhistidine. Muscle protein loss, a response to a reduction in RNA, is also reversable. A head-down tilt for 7-14 days results in diuresis and natriuresis. These changes are reversed within 24 hours after removal from the restraint harness. Physiological effects of suspension restraint can be used to simulate and predict responses to microgravity exposure.

INTRODUCTION

Antiorthostatic hypokinetic (AOH) suspension in growing rats results in several physiological changes which may be separately credited to positioning (head-down tilt, 15-20°) and to hypokinesia by removal of load bearing function in the hind limbs. Details of the suspension system were reported (1) and an explanation of antiorthostatic positioning was provided (2).

RESULTS AND DISCUSSION

Results of one and two week suspensions and recovery are summarized in Table 1. Suspension is accompanied by an initial loss of weight and a slowing of weight gain during the one and two week period. The extent of muscle atrophy differs in the four muscles measured: soleus>gastrocnemius= plantaris>extensor digitorum longus (EDL). The soleus, chiefly a slow twitch, load bearing antigravity muscle lost 35% of its mass in one week and 45% in two weeks of suspension. The gastrocnemius and the plantaris, both with mixed fiber composition, showed a similarity of response in one week (22%-24% loss) and thus can be credited with limited antigravity function. The EDL, composed chiefly of fast twitch fibers, showed little or no change in muscle mass during one and two weeks of suspension. The EDL, therefore, is not considered critical in load bearing functions.

Reductions of muscle mass are paralleled by changes in protein content. Loss of muscle protein was not accompanied by reduced muscle DNA content. Muscle RNA content, however, correlated well with the extent of atrophy. This is indicative of a reduction in the size of the muscle cells rather than a reduction in their number. Presumably, therefore, muscle atrophy results from decreased protein synthetic capacity. There were marked increases in both muscle mass and RNA content upon removal from suspension.

Further evidence for muscle catabolism was found in the increased levels of excretion of nitrogenous end products (urea, NH_3, 3 methylhistidine) during hypokinesia, and the recovery to control levels after removal from suspension. Increased levels of urinary K^+ also argued for intracellular breakdown rather than reduction in numbers of muscle cells. The general conclusions are that differential muscle atrophy results from hypokinesia and that recovery occurs after seven or 14 days of suspension hypokinesia.

The suspended rat in a head-down tilt position for seven and 14 days, as contrasted to the suspended animal in horizontal position (2), showed marked diuresis and natriuresis. When removed from suspension at either seven or 14 days, there is a marked and immediate reversal of the fluid and electrolyte excretion patterns.

CONCLUSIONS

We are able to conclude that suspension hypokinesia results in muscle atrophic changes which are in concert with responses seen in rats in true weightlessness in COSMOS 605, 690 and 936 experiments (3, 4 and 5). In our preparation the hindlimbs are free hanging but non-load bearing. Cast immobilization and small cage restraint are also useful approaches to hypokinetic and hypodynamic responses. In our opinion, the suspension system compares more directly with animals and humans in weightless conditions because the rats continue to experience limb mobility in the absence of load bearing functions.

The antiorthostatic positioning of head-down tilt in the rat changes the normal gravitational vector. The blood volume and electrolyte shifts, as seen in diuresis and natriuresis, are comparable to those obtained with other laboratory procedures (i.e., prolonged bed rest, water immersion, inflation of intracardiac balloon catheters and negative-pressure breathing; reviewed earlier (2). In our preparation we assume an expanded thoracic blood volume in which the cardiopulmonary stretch receptors sense a change in blood volume. Thus, this preparation lends itself to experiments which

mimic weightlessness by the removal of gravitational influences from the cardiovascular system.

The model provides an experimental approach to two variables seen in present and future space experiments, namely hypokinesia (and/or hypodynamia) and antiorthostasis.

Supported by NASA Grants NSG-2191 and NSG-2325.

REFERENCES

1. Musacchia, X. J., D. R. Deavers, G. A. Meininger and T. P. Davis. 1980. A model for hypokinesia: effects on muscle atrophy in the rat. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 48: 479-486.
2. Deavers, D. R., X. J. Musacchia and G. A. Meininger. 1980. Model for antiorthostatic hypokinesia: head-down tilt effects on water and salt excretion. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 49:576-582.
3. Ilyina-Kakueva, E. I., V. V. Portugalov and N. P. Krivenkova. 1976. Space flight effects on the skeletal muscles of rats. Aviat. Space Environ. Med. 47(7): 700-703.
4. Ilyina-Kakueva, E. I. and V. V. Portugalov. 1977. Combined effect of space flight and radiation on skeletal muscles of rats. Aviat. Space Environ. Med. 48(2): 115-119.
5. Chui, L. A. and K. R. Castleman. 1980. Morphometric analysis of rat muscle fibers following space flight and hypogravity. The Physiologist. 23: Suppl., S-76-78.

TABLE 1

Physiological Alterations Induced by Antiorthostatic Hypokinesia (AOH) in Rats (Sprague Dawley, males)

	AOH Suspension			Recovery After	
Time	1-2 Days	1 week	2 weeks	1 week	2 weeks
Experimental parameters*					
Body weight	↓	(1)	(1)	↓(2)↑	↓(2) ↑
Muscle weights					
Soleus	↓	↓	↓	↑(3)	↑(4)
Gastrocnemius	↓	↓	↓	↑(5)	↑(5)
Plantaris	↓	↓	↓	↑(5)	↑(5)
Extensor digitorum longus	••	••	••	••	••
Nitrogenous excret.					
Urea	↑	↑	↑	↓	↓
Ammonia	↑	↑	↑	↓	↓(6)
3-methylhistidine	↑	↑	↑	••	••
Urine Volume	••	↑	↑	↓	↓(7)
Electrolyte					
Na^+	↓	↑	↑	↓	↓(7)
K^+	↓	↑	↑	↓	↓(7)
Plasma Osmolarity	••	••			

*All parameters are compared to age matched metabolism cage controls (MCC), except muscles which are compared to both MCC and to weight matched controls (WMC)

↓ = decrease ↑ = increase •• = no difference

(1) After 2 days AOH, there is no further loss in body weight
(2) After 1 wk, body weight increasing but less than MCC
(3) After 1 wk, weight is comparable to 1 wk MCC
(4) After 2 wks, weight is increasing but less than 1 wk MCC
(5) After 1 and 2 wks, weight is increasing but less than 1 wk MCC
(6) After 2 wks rate of recovery is slowed
(7) After 2 wks, amount excreted is initially reduced but remains slightly elevated

Symposium on Gravitational Physiology
Chairman's Introduction

Thora W. Halstead

Office of Space Science
National Aeronautics and Space Administration
Washington, D.C. 20546

Although the biological effects of gravity have been acknowledged since Galileo's time, gravitational physiology is in its infancy. The inability to control gravity on earth - to remove its effect for any significant period of time - has not only limited research, it has scientific interest in this area. Gravity could be the prime environmental factor molding and controlling life on earth, yet we do not even know the importance of gravity to sustaining life itself.

While gravitational physiology encompasses the physiological responses to the full range of gravitational force both above and below earth's norm of one, weightlessness has been the most difficult condition to either simulate or achieve. The advent of aviation and especially high-performance aircraft early in this century stimulated an interest in the physiological changes produced by increased force environments. This in turn fostered centrifuge research to simulate high gravity conditions. With the birth of the space age about 25 years ago, the opportunity for experimentation over the full spectrum of G became a reality. Ready access to this new zero-G environment has not yet occurred, but the opportunities to test and validate hypotheses developed through ground based research are now within our grasp. Systems and models designed to simulate weightlessness can be validated by comparison of ground and flight experimental results. The increasing opportunities for experimentation in space paired with the costliness of spaceflight presents a strong argument for the development of hypotheses and substantial data gathering on the ground prior to flight.

Research in the past has focused on the adverse physiological effects resulting from exposure to altered gravity. There has been a continuing need to know these effects if man is to survive in space for prolonged periods of time. Surmounting these obstacles to space habitation will also require ground based research, but of an applied nature in which science serves space. Gravitational physiology will truly come of age when gravity and space are used primarily for research that addresses fundamental issues in biology and medicine and answers questions of intrinsic scientific interest. When gravity and space clearly demonstrate their usefulness as biological tools they will attract scientific interest. The value of gravity as the ultimate tool with which to investigate the physiology of the vestibular system, to learn how organisms sense gravity and the mechanisms by which they transmit this information to evoke a response, to probe the formation of structural elements such as lignin and bone-calcium, and to unravel the secrets of early development needs to be recognized by the biological community at large.

Papers to be presented in this Symposium on Gravitational Physiology and during the entire IUPS Commission on Gravitational Physiology Program demonstrate the potential before us to use space experimentation to validate and refine ground based research. The opportunities for flight will always be limited, but we now have the opportunity for unlimited ground based gravitational research. Many approaches are available to use gravity as a tool to understand the physiology of living organisms on earth as well as the effects of the space environment on biological processes. Gravitational forces of greater and less than one can be simulated through the use of centrifuges, clinostats and systems that induce hypodynamia, hypokinesia and/or antiorthostasis. Mutant organisms are also available with defective G sensing systems.

The future of gravitational physiology is as bright as we make it. Neither physicists nor engineers question the importance of gravity to the world in which we live. Geophysics, solar physicists, planetary scientists and astronomers have eagerly made space their remote workshop. The biological community, however, has not yet recognized the value of gravity as a tool and space as a laboratory. The participants of this meeting are among the pioneers in gravitational physiology; they need to be the missionaries as well.

OPENING REMARKS

H. Bjurstedt
Chairman, IUPS Commission on Gravitational Physiology

Department of Environmental Physiology
Karolinska Institutet, Stockholm, Sweden

Ladies and Gentlemen,

On behalf of the International Commission on Gravitational Physiology I wish to welcome all of you to this international Symposium, the third of its kind. Not long ago, in 1978, the Commission met here in Innsbruck in conjunction with the 21st COSPAR Plenary Meeting. On this occasion a 2-day Symposium on Gravitational Physiology was held under the joint sponsorship of COSPAR, the International Union of Physiological Sciences, the International Academy of Astronautics, and the International Association for Plant Physiology. The subject field of the Commission had already attracted increased interest from the international scientific community, and in recognition of this a number of decisions was taken at the 1978 Innsbruck meeting in order to step up the Commission's activities. Of prime importance for the continued activities of the Commission was the decision to hold annual meetings, intended to invite participation from all scientists who wish to communicate research results in gravitational physiology. Another decision was to expand the membership of the Commission, which at present includes representation from human and mammalian physiology and disciplines concerned with the physiology of plants and with cell and developmental biology.

It is a great pleasure for me to express the Commission's gratitude to the German and Austrian Physiological Societies for graciously agreeing to act as hosts for our Commission's Third Annual Meeting, of which this Symposium is part. I wish, in particular, to convey the Commission's appreciation to Professor P. Deetjen for his personal contributions to the many tasks involved in the preparations and realization of our Meeting here in this historical and beautiful city.

Gravitational physiology is a branch of the physiological sciences which is concerned with the effects of the force of gravity, as well as increases and decreases thereof, on basic mechanisms responsible for the origin, development and progression of life. There is ample evidence that changes in magnitude and direction of this force influence function in living matter at numerous levels, from simple cells to organs and organ systems in the complicated human being. To what extent and how the normal force environment influences function in living matter has only relatively recently become a matter of systematic experimental study. Only the advent of the space age made it possible, for the first time in man's history, to expose living matter to protracted weightlessness, arising from the absence (or more strictly speaking, the neutralization) of earth's gravity, in orbiting and coasting space vehicles. The newly found access to the 'gravity-free' state, and the possibility of manipulating the gravity factor from its norm of 1 down to zero, suddenly provided a powerful tool for biological and medical investigations of fundamental interest.

As is evident from today's program, this Symposium is devoted to a variety of problems concerned with the effects of the force environment on function and morphology. The topics include graviperception in animals and plants, high-G effects on the circulatory system in humans, physiological effects of restraint in animals, metabolic scale effects in mammals, and models for simulating weightlessness. Our Second Annual Meeting held during the 1980 International Physiological Congress in Budapest included a symposium with results from the Cosmos 1129 joint USSR/USA spaceflight experiments as its main theme. The present Symposium will highlight additional results from these experiments and will also review results from prolonged manned flights onboard the space station Salyut-6. Our present Meeting also includes 8 sessions with voluntary papers in gravitational physiology, the first 4 of which took place yesterday, with another 4 sessions scheduled for tomorrow. The Commission notes with great satisfaction the continued interest shown in our meetings by scientists in many branches of physiology.

ROOT CELL GRAVIREACTION : HORMONE INTERACTION

P.E. Pilet

Institute of Plant Biology and Physiology of the University
1005 Lausanne, 6 Pl. Riponne, Switzerland

ABSTRACT

Two endogenous hormones, Indole-3yl-acetic acid (IAA) and Abscisic acid (AbA), interfer in the elongation zone of gravistimulated maize roots, inducing a downward curvature (positive gravitropism).

INTRODUCTION

The gravity-dependent orientation of roots can be regarded as a chain of several events.

At first the graviperception corresponds to the direct action of the gravity stimulus on some cell receptors in the root cap, inducing therein some physical changes which are "transformed" into biochemical information.

Then the regulators thus formed are transmitted to the extension root zone (site of the gravireaction) which grows asymmetrically to produce downward curvature (3).

In the present report, some interactions between two of these regulators (indol-3yl-acetic acid : IAA and abscisic acid : AbA) will be briefly discussed.

MATERIAL AND METHOD

Several cultivars of Zea mays were used. Selected caryopses were grown in darkness according to a method previously described (5). Primary roots were used (intact or segments). Gravicurvature was recorded by making shadowphotographs. Techniques of GC-MS analyses were described elsewhere for IAA (9) and AbA (8).

RESULTS AND DISCUSSION

Several topics will be discussed here : gravireaction; IAA and AbA content; involvement of IAA; involvement of AbA; endogenous AbA redistribution; IAA and AbA interactions.

Gravireaction

As can be seen in Table 1, the gravicurvature of intact roots was about two times larger than that of apical root segments and the white light similarly enhanced these reactions (1).

IAA and AbA content

Data reported in Table 2 indicate that both IAA and AbA were present in growing maize roots : the largest level being obtained for IAA in the cap (9) and for AbA in the apex (5,8).

TABLE 1 : Gravireaction in terms of downward curvature (degrees ± standard error) after 6 h of horizontally placed maize (cv.LG 11) roots (intact roots or apical root segments) maintaines in the dark or in white light.

	Dark	White light
Intact roots	39.8 ± 2.0	52.0 ± 2.1
Apical root segments	20.8 ± 1.0	32.4 ± 1.8

TABLE 2 : IAA and AbA content (in ng per kg of fresh weight)

Analyses for different regions of the primary maize (cv.Kelvedon) roots

mm from the tip	IAA	AbA
0.0 - 0.5	356.6 ± 16.2	36.1 ± 7.6
0.5 - 1.0	179.9 ± 23.4	66.5 ± 18.4
1.0 - 4.0	76.5 ± 28.3	33.3 ± 0.9

Involvement of IAA (7)

Gravireaction of root segments is given in Figure 1. Apical root segments showed a strong curvature (A) while no reaction was obtained for detipped segments (B) unless the tip was replaced (C).

After the endogenous IAA was reduced (exodiffusion pretreatment), detipped segments (D) still showed no gravireaction, while the curvature of retipped segments (E) was lower than that of segments C.

These data were compatible with the view that the reduction of endogenous IAA caused a decrease in gravireactivity. In fact, as indicated in Figure 2, a IAA treatment may stimulate or reduce - according to the concentrations used - the root gravitropism.

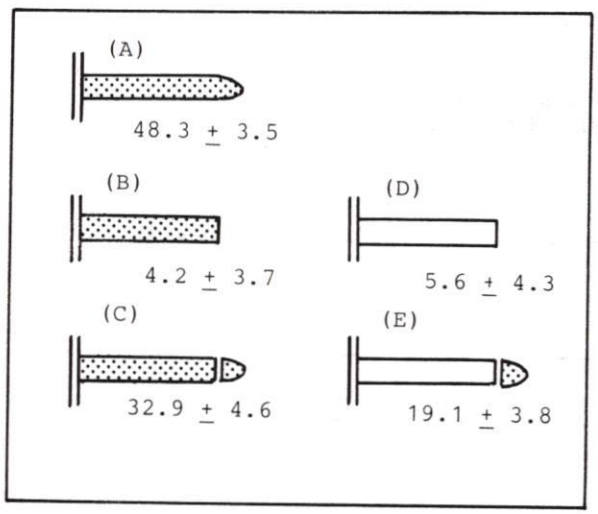


FIGURE 1 : Downward curvature (degrees ± standard error) after 7 h of horizontally placed maize (Orla) apical root segments, with moist buffered (pH 6.1) filter paper on their basal cut end. Exodiffusion pretreatment (D,E): before the gravistimulation, decapitated segments were kept vertical in darkness with their apical cut surface placed for 4 h on buffered (pH 6.1) agar. Segments not to scale.

Involvement of AbA

This hormone caused an inhibition of root elongation (6).

On the other hand, when applied to the very tip (Fig. 3), AbA - for all the tested concentrations- induced a significant increase in the gravireaction of the root segments (2).

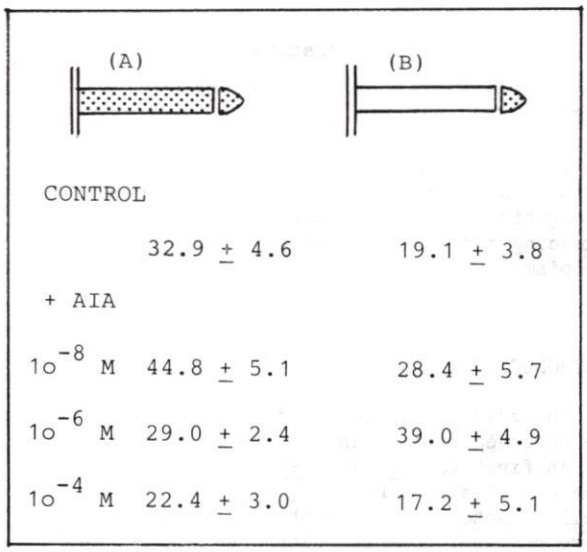


FIGURE 2 : Downward curvature (degrees ± standard error) after 7 h of horizontally placed maize (Orla) apical root segments, with moist buffered (pH 6.1) filter paper containing or not containing IAA, on their basal cut ends. Segments not to scale.

Clearly, these data support the view that AbA is one of the endogenous growth inhibitors which have been shown (5) to be formed or released in the cap cells.

Endogenous AbA redistribution

Results (8) presented in Table 3 indicate that when gravistimulated roots bend downward, the AbA content was significantly higher in the lower halves of the growing zone than in the upper halves. In contrast for the roots displaying no graviresponses or negative curvature, no significant differences can be found.

But it has to be said that for 100 % of roots tested, 53.2 % correspond to the roots having a positive gravireaction, 39.3 % to roots without any gravicurvature and 7.5 % to roots showing an upward reaction.

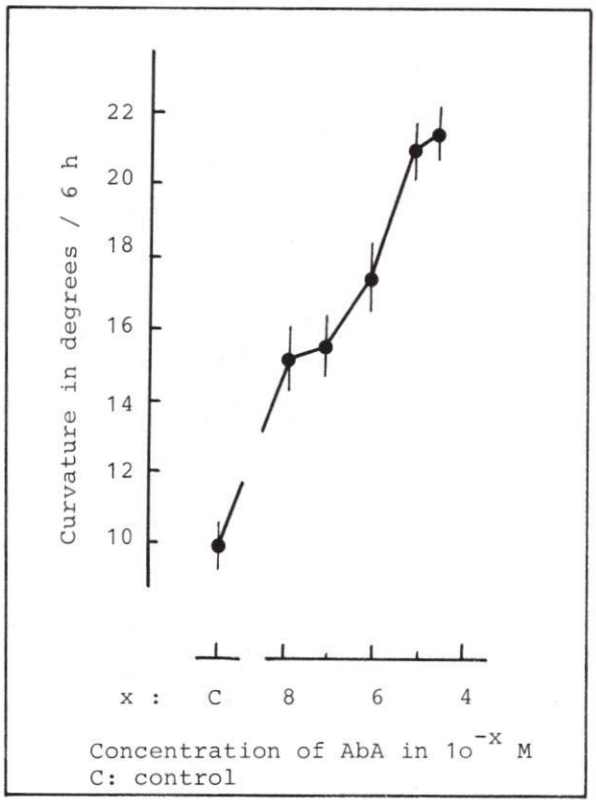


FIGURE 3 : Downward curvature (degrees ± standard error) after 6 h of horizontally placed maize (cv.LG 11)apical root segments (darkness). AbA pretreatment: intact seedlings maintained vertically 2 h with a droplet on the tip of primary roots. 5 ul droplet of a buffered solution (3,3-DGA,1o^{-3} M; pH 6.0) with or without AbA at different concentrations.

TABLE 3 : AbA content (ng ± standard error / 100 segments) in the 4 mm maize (cv.LG 11) root tips from which the first mm has been removed. Tips were longitudinally split into 2 equal halves Intact segments (10 mm) first maintained 2 h in horizontal position (white light)

Reaction*	upper halves	lower halves	total
+	16.2 ± 1.4	20.8 ± 1.8	37.0 ± 3.2
	44 %	56 %	100 %
o	19.1 ± 1.4	20.9 ± 0.5	40.0 ± 1.9
	48 %	52 %	100 %
-	18.9 ± 0.7	17.1 ± 1.5	36.0 ± 0.8
	53 %	47 %	100 %

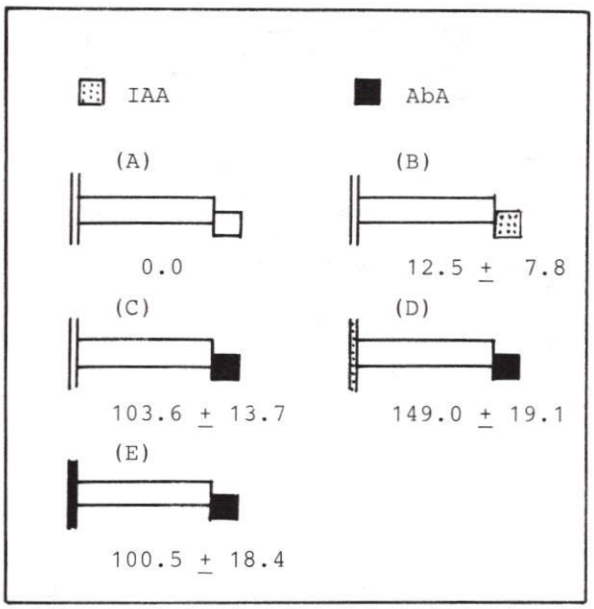


FIGURE 4 : Downward curvature (relative values ± standard error) after 7 h of horizontally placed maize (Kelvedon) apical root segments decapitated at 0.6 ± 0.1 mm, with moist buffered (pH 6.1) filter paper on their basal cut section and a agar block half covering their apical cut end. Filter paper and agar block with or without IAA or AbA at lo^{-6} M. Segments not to scale.

IAA and AbA interactions

Some experiments are summarized in Figure 4. When IAA was asymmetrically given on the apical cut end of apical root segments in horizontal position, no significant curvature was obtained (B), while AbA induced a strong reaction (C) stimulated by a basal application of IAA (D) but not by similar treatment with AbA (E). These data (4) confirm the preferential transport of these two regulators which interfer when controlling root gravireactivity.

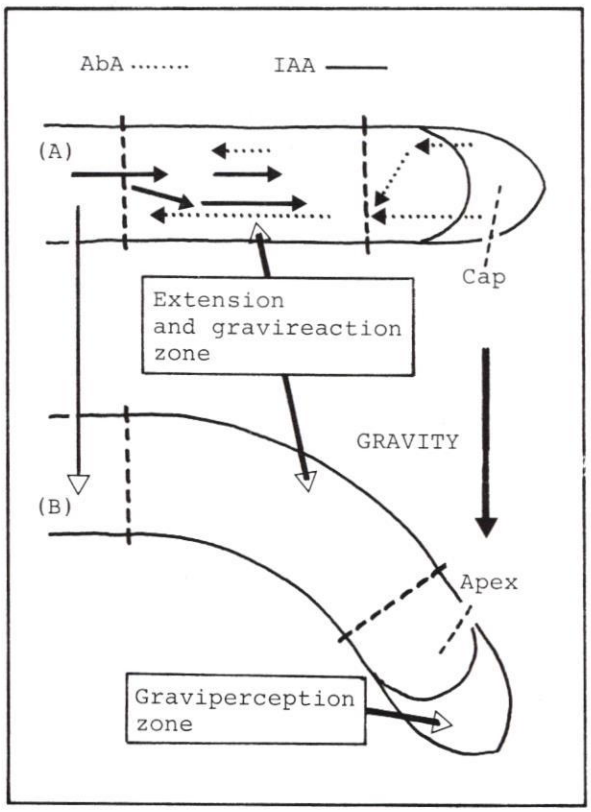


FIGURE 5 : Diagram showing the two preferential hormone (IAA and AbA) flows inside the maize root tip gravistimulated (A) and then gravireacting (B). Graviperception located in the root cap and gravireaction (gravitropism +) in the elongation zone.

CONCLUSION (Figure 5)

Growth reaction of gravistimulated roots is regulated at least by the IAA movement which is strongly acropetal and by the AbA transport preferentially basipetal.

These two hormones being in larger amount in the lower part of the growing zone induce a growth inhibition of this region, causing a downward root curvature.

REFERENCES

1. Beffa, R. and P.E. Pilet (in press). Elongation and gravireaction of intact and segmented roots : light effects. Physiol. Plant.

2. Chanson, A. and P.E. Pilet. 1981. Effect of abscisic acid on maize root gravireaction. Plant Sci. Lett. 22:1-5.

3. Juniper, B.E. 1976. Geotropism. Annu.Rev.Plant Physiol. 27:385-406.

4. Pilet, P.E. 1975. Abscisic acid as a root growth inhibitor : physiological analyses. Planta 122:299-302.

5. Pilet, P.E. 1977. Growth inhibitors in growing and geostimulated maize roots. In Plant Growth Regulation (P.E. Pilet, ed.) Springer Verlag, Berlin New York, pp. 115-128.

6. Pilet, P.E. and A. Chanson. 1981. Effect of abscisic acid on maize root growth. A critical examination. Plant Sci. Lett. 21:99-106.

7. Pilet, P.E. and M.C. Elliott. 1981. Some aspects of the control of root growth and georeaction: the involvement of IAA and AbA. Plant Physiol. 67:1047-1050.

8. Pilet, P.E. and L. Rivier (in press). Abscisic acid distribution in horizontal maize root segments. Planta

9. Rivier, L. and P.E. Pilet. 1974. Indolyl-3-acetic acid in cap and apex of maize roots : identification and quantification by mass fragmentography. Planta 120:107-112.

THE EFFECT OF GRAVITY ON THE DISTRIBUTION OF PLANT GROWTH SUBSTANCES IN PLANT TISSUES

Wolfram Hartung

Lehrstuhl Botanik 1, Universität Würzburg, Mittlerer Dallenbergweg 64
D 8700 Würzburg, Germany

ABSTRACT

The distribution of abscisic acid(ABA) in root tip tissues and of indole acetic acid (IAA) in shoot tissues of several plants is not affected by gravity.Pretreatment with ABA (roots) and IAA (shoots), however, increases the geotropic reaction of the organs.Treatment of the roots and shoots that alters the pH-gradients within the cellsand thus the cellular phytohormone distribution reduces the sensitivity of horizontally placed plant organs to gravity. The results communicated in this paper cast doubt on the validity of the Cholodny Went theory. It appears possible that gravity influences the cellular compartmentation of plant growth substances.

INTRODUCTION

It is well established that plant growth substances, especially ABA (roots) and IAA (stems) are involved in the geotropic reaction of plants (3,7,9,10,13). ABA and IAA are present in the organs concerned, asymmetric application of ABA to roots and IAA to stems induces bending of the plant organs. However, it is still not understood how endogenous growth regulators are translocated to the cells involved in the geotropic reaction. According to the Cholodny Went theory ABA and IAA are transported laterally to the lower halves of horizontal roots and stems. There are data in the literature which support this hypothesis (10,13). However, several authors were unable to find an asymmetric distribution of exogenously applied radioactive phytohormone under the influence of gravity (4, 7,9). We have reinvestigated this problem, mainly measuring the distribution of ABA in horizontal roots of intact runner bean-, broad bean- and maize seedlings.

METHODS

Seedlings of Phaseolus coccineus(cv.Weißer Riese, 5 days old), Vicia faba (6 days old) and Zea mays (cv. Forla and Blizzard, 4 days old) were cultivated in the dark at 22°C in moist vermiculite. 2.2 pmoles of (^{3}H)ABA (specific activity 18 Ci mmol^{-1}, Radiochemical Centre Amersham) were applied in an aqueous solution of 1 µl to the extreme tip of vertically placed roots of intact seedlings. After 30 min. exposure in the dark in water saturated atmosphere excess ABA was removed by washing. Up to 0.3 pmoles (^{3}H)ABA per g fresh weight were taken up by the root tips during the incubation period. The endogenous hormone balance of the root tissue is not altered by these very small amounts of ABA. Afterwards roots were placed horizontally or vertically (controls) in the light (5 klx) or in the dark at 22°C in a controlled temperature cabinet. After 1 and 2 h, shortly before geotropic bending becomes clearly visible, two segments (5 mm long) were cut from the apical end of the root. They were divided in two halves as shown in Fig.1 and their radioactivity was counted in a ß-scintillation counter.DPM originating from ABA were expressed on fresh weight basis. Curvature experiments were carried out in the light with 1 day old Lepidium sativum seedlings. Plant hormones

were incubated for 20 min. to the roots or hypocotyls. pH-gradients were abolished by preincubation with 5 mM KNO_2 in 25 mM MES buffer, pH 5 for 20 min.(8,11). 4 h after nitrite treatment no effects on root growth could be observed. Nitrite could be removed from the tissue by washing in 25 mM HEPES buffer pH 7.8, three times for 10 min.
Growth curvatures and elongation growth were recorded as described by Phillips and Hartung(9).

RESULTS

Fig.1 shows the distribution of tritiated ABA in horizontally and upright placed root tips 1 h (A) and 2 h (B) after ABA application in the light.

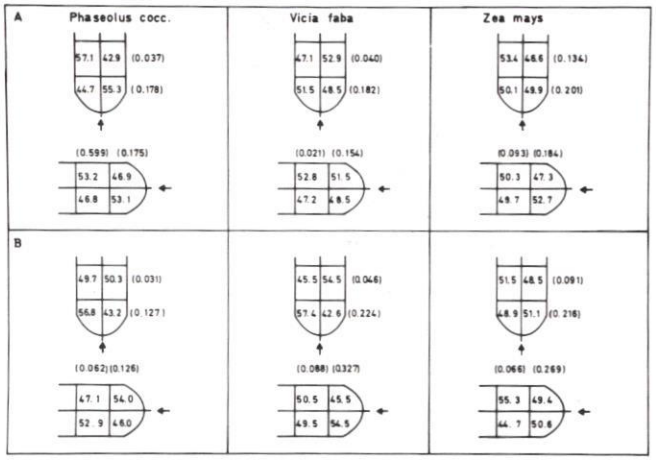


Figure 1 Lateral distribution of radioactivity in the two apical 5 mm long segments of root tips of intact seedlings kept in either horizontal or upright position for 1 h (A) or 2 h (B) after application of 2.2 pmoles of tritiated ABA to the extreme root tip (arrow) in the light.The figures in brackets show the amount of radioactive ABA taken up by the corresponding root segments (pmoles g fresh weight^{-1}). The distribution is expressed as percentage of total radioactivity present in the opposite lateral halves of each portion of segment.Similar ABA distribution patterns could be observed when experiments were performed in darkness.

After 2 h the root tips of horizontally placed seedlings began to bend. According to the Cholodny Went theory an asymmetric distribution of ABA should occur in geotropically stimulated root tips within 2 h. After exogenous application ABA was on the other hand able to enhance the geotropic response of the root tips (Fig.2).

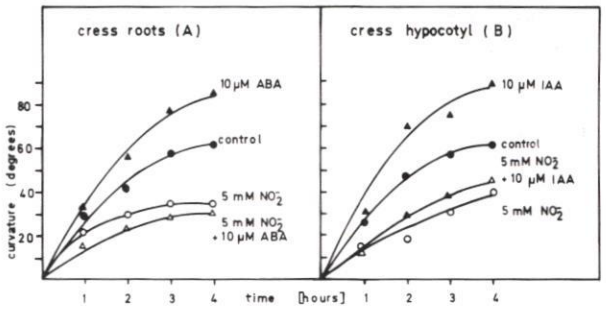


Figure 2 Curvatures of horizontally placed cress roots (A) and cress hypocotyls (B), dependent on preincubation with 10 µM ABA (A) or 10 µM IAA (B) ± 5 mM KNO_2 in the light.Elongation growth of nitrite treated seedlings was not significantly different from the controls.

Incubation with 5 mM KNO_2 at pH 5 inhibited the ABA induced bending of cress roots, but not the elongation growth of the root tips (not shown). When nitrite was washed away by alkaline HEPES buffer, ABA effects appeared again(not shown). Identical results were obtained with the cress hypocotyl and IAA (Fig. 2B).

DISCUSSION

ABA applied in pmol quantities to the root tip of horizontally placed seedlings of Phaseolus coccineus, Vicia faba and Zea mays is not translocated laterally within 2 h neither in the dark nor in the light. These results confirm and extend earlier findings of Hartung (7), who applied (^{14}C)ABA asymmetrically in higher concentrations directly behind the root cap of Phaseolus coccineus with micropipettes and found as long lateral transport until an equal distribution was reached. Suzuki et al. (12) and Weiler (personal communication) analysed endogenous ABA of Zea roots in the upper and lower halvesafter geotropic stimulation and also could not find any asymmetric distribution of ABA in root tips. On the other hand ABA treatment of root tips increases the geotropic reaction (3, and Fig. 2A) which indicates that ABA is involved in the georeaction.

We have a similar situation with green tissues of dicotyledonous plants (4,9). IAA, whose rôle as a regulator of geotropic reaction in stems appears established, is not translocated laterally in sunflower internodes within 6 h, but enhances also the sensitivity of the organs for gravity if applied exogenously.

These results do not agree with the postulates of the Cholodny Went theory. The only plant organ where gravity induced lateral IAA transport has clearly been demonstrated is the grass coleoptile (9,13). In this case, however, very often the asymmetric hormone distribution develops rather late, after the bending of the coleoptile. Digby and Firn (4) have therefore suggested that in coleoptiles hormone asymmetry is a consequence rather than a prerequisite of the growth curvature.

They believe also that lateral redistribution depends on a lateral pH gradient developing after gravity induced bending in the grass coleoptile tissue, originating from different respiratory activity in upper and lower halves of horizontal coleoptiles.

The question to be solved is now how IAA and ABA can develop a greater growth activity in the convex sides of root and stem tissues without being laterally transported.

Possibilities to reconcile this contradiction could be different activities of metabolism, synthesis and longitudinal transport between the upper and lower halves of geotropically responding tissues, as pointed out earlier (9).

I would like to propose the explanation that phytohormone compartmentation is affected in geotropically reacting organs.

We know that the intracellular distribution of ABA and IAA, which are weak acids (pK=4.75), between the organelles of a cell depends on the pH gradients between the cellular compartments. Protonated ABAH and IAAH penetrate the biomembranes easily and are trapped according to the Henderson-Hasselbalch equation in the more alkaline compartments (cytoplasm, chloroplast stroma) as anions ABA^- and IAA^-(1,8). Change of pH in one compartment (by illumination or osmotic stress in the stroma) alters the hormone distribution in the cells and influences the hormone transport in the symplastic and apoplastic ways (2,8 and Hartung, unpublished).

The experiments of Fig.2 show that the geotropic reaction takes place only if the pH gradients in the cell and thus the hormone compartmentation is not disturbed by the nitrite treatment.

On the other hand gravity induces H^+extrusion from the convex side of horizontal stems (5) which could change the proton and phytohormone distribution in the cells of geotropically reacting tissues. Additionally Wordekemper and Schlack (14) observed that phototropism of rice seedlings was eliminated under zero gravity conditions in skylab. Phototropism, however, is believed to be strictly dependent on a functionating IAA transport system and thus on pH gradients (6) which also control hormone compartmentation.

It is concluded therefore that, in the case of roots for instance, ABA would be produced in the root cap, transported to the elongation zone uniformly where gravity could differentially influence the compartmentation on the upper and

and lower side without affecting the uniform distribution within the tissue.

ACKNOWLEDGEMENTS

I am grateful to Dr.W.Kaiser for stimulating discussions, to Miss A.Weber and Mr.V.Kummer for expert assistance and to Deutsche Forschungsgemeinschaft for financial support.

REFERENCES

1. Behl,R., Jeschke,W.D. and Hartung,W. (in press). A compartmental analysis of abscisic acid in roots of Hordeum distichon.J.exp.Bot.
2. Cowan,I.R., Hartung,W., Raven,J.A. and Farquhar,G.D.(in press). A possible role for abscisic acid in coupling stomatal conductance and photosynthetic carbon metabolism in leaves. Austr.J.Plant Physiol.
3. Chanson,A. and Pilet,P.E. 1981. Effect of ABA on maize root gravireaction. Plant Sc.Letters 22,1-5.
4. Digby,J. and Firn,R.D. 1976. A critical assessment of the Cholodny Went theory of shoot geotropism. Curr.Adv.Plant Sc.8,953-960.
5. Evans,M.L., Mulkey,T.J. and Kuzmanoff,K.M. 1981. Directional H^+-efflux during shoot geo- and phototropism.Plant Physiol. Suppl.67,99.
6. Goldsmith,M.H.M. 1977. The polar transport of auxin.Ann.Rev.Plant Physiol.28,439-478.
7. Hartung,W. 1976.Der basipetale Abscisinsäuretransport in Wurzeln intakter Bohnenkeimlinge und seine Bedeutung für den Wurzelgeotropismus.Planta 128,59-62.
8. Kaiser,W.R. and Hartung,W. 1981. Uptake and release of abscisic acid by isolated photoautotrophic mesophyll cells, depending on pH gradients.Plant Physiol.68,202-206.
9. Phillips,I.D.J. and Hartung.W. 1976.Longitudinal and lateral transport of (3,4-^{3}H) gibberellin A_1 and 3-indolyl(acetic acid-2-^{14}C) in upright and geotropically responding green internode segments from Helianthus annuus. New Phytol.76,1-9.
10. Pilet,P.E. 1976. Growth inhibitors in growing and geostimulated maize roots. In Pilet(Ed.) Plant growth regulation, Springer,Berlin Heidelberg,New York, pp.115-128.
11. Purczeld,P., Chon,C.J., Portis,A.R., Heldt,H.W., and Heber,U. 1978. The mechanism of the control of carbon dioxide fixation by the pH in the chloroplast stroma. Studies with nitrite mediated proton transfer across the envelope. Biochim.Biophys.Acta 501,488-498.
12. Suzuki,T., Kondo,N and Fujii,T. 1979.Distribution of growth regulators in relation to the light induced geotropic responsiveness in Zea roots. Planta 145,323-329.
13. Wilkins,M.B. 1979. Growth control mechanism in gravitropisms.Encycl.Plant Physiol. New Series. 7,601-626.
14. Wordekemper,J.G. and Schlack,D.W. 1979. cited by Kessler,J.O. 1979.Gravity sensing, polar transport and cytoplasmic streaming in plant cells. The Physiologist 22,S47-S48.

INVOLUNTARY AND VOLUNTARY MECHANISMS FOR PREVENTING CEREBRAL ISCHEMIA DUE TO POSITIVE (G_z) ACCELERATION

E. H. Wood, E. H. Lambert, and C. F. Code

Department of Physiology and Biophysics
Mayo Medical School
Rochester, Minnesota 55905

INTRODUCTION

Positive, G_z, acceleration produces a severe stress on the circulatory system. The magnitude of this stress imposed in the sitting position similar to that of a pilot of a fighter plane is shown diagrammatically in Figure 1. Note that maintenance of an arterial pressure of zero at the base of the brain during an exposure to +5 G_z requires a pressure of 370 mm of Hg at foot level and, in a relaxed subject, a venous pressure of 250 mm of Hg would be needed to maintain venous return from the feet to the base of the heart.

This suggests that venous return to the heart would practically cease during an exposure to +5 G_z, so that a dramatic fall in blood pressure at heart level would follow.

Photokymographic recordings (Figure 2) during 30 second exposures of a healthy, relaxed human to +4 and +5 G_z on the Mayo Human Centrifuge in the mid 1940s illustrate that in fact not only is arterial pressure maintained at heart level, but amazingly, after the initial 5 seconds, an involuntary increase in arterial pressure to nearly 200 mm of Hg occurred. This degree of hypertension was sufficient to push blood back up to the brain and retina and restore vision during a sustained exposure to a force environment 5 times greater than planet Earth.

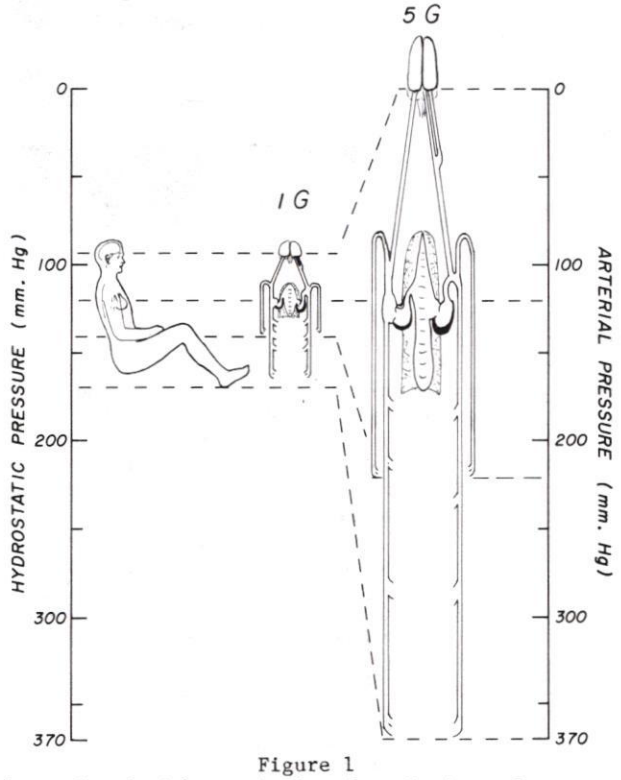


Figure 1

(Reproduced with permission from Wood et al., Fed. Proc. 22(4):1024-1034 (July-August) 1963.

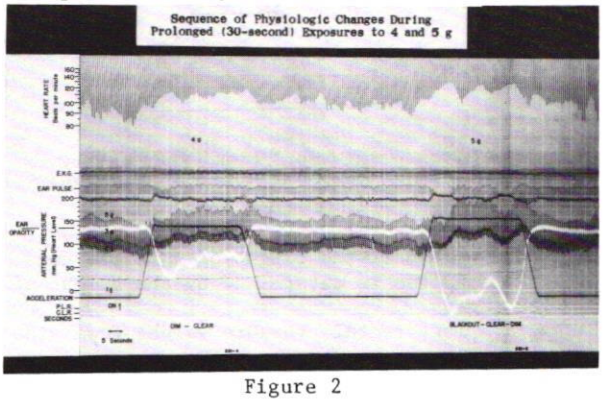


Figure 2

How is this amazing involuntary compensatory capability of the human cardiovascular system accomplished?

There are at least two major factors: the first, based on the physical characteristics of the human thoracic cage-abdominal system and the second on autonomically mediated baroreceptor reflexs.

The major physical factor is illustrated in the bottom tracing of multiple variables recorded from a healthy human during an exposure to 4.5 G maintained for 15 sec (Figure 3). This recording of pressure from an intrarectal balloon is an indirect measure of intra-abdominal pressure, i.e., the pressure in the peritoneal fluid in the dependent regions of the abdominal cavity.

Note that this pressure of about 25 mm Hg recorded at 1 G which is sufficient to maintain venous return to the heart, increases in direct proportion to the level of acceleration so that at 4.5 G, the intra-abdominal pressure of about 125 mm Hg is sufficient to maintain venous return from all the abdominal organs and hence, it is not too surprising that arterial pressure is maintained during such an exposure.

In other words, as first pointed out by Rushmer (1), the abdominal contents behave like a hydrostatic system, that is, as if the organs were immersed in a bucket of water, the upper surface of which is at the diaphragm. As this recording illustrates, variations in the height of the diaphragm with each respiratory cycle produce corres-

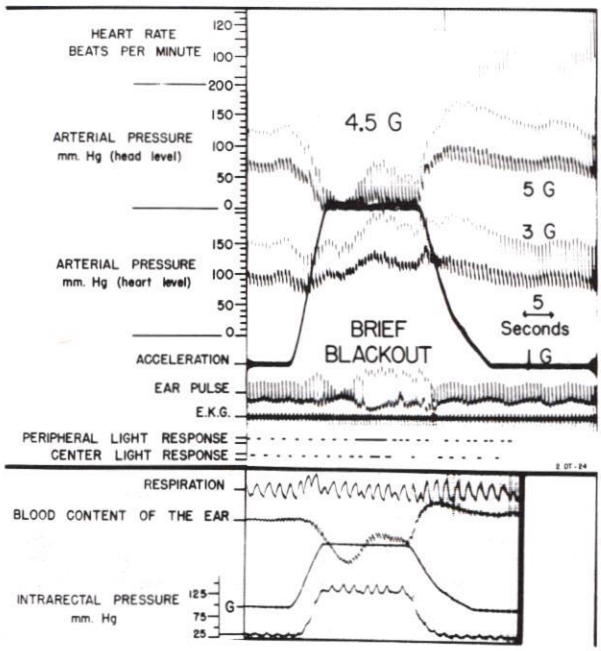


Figure 3

(Reproduced with permission from Wood et al., Fed. Proc. 22(4):1024-1026 (July-August) 1963.

ponding changes in intra-abdominal pressure as would be expected if the height of this presumed hydrostatic system is determined by the height of the diaphragm.

Average intrarectal pressure values from nine subjects during 15 second exposures to up to 5 G_z are shown in Figure 4. The dashed line indicates the values which would be expected if the abdominal cavity were filled with water up to the height of the diaphragm. Such a water-filled system would afford perfect protection to the abdominal circulation with no decrement in venous return to the heart. The fall off from the predicted level for perfect protection at 4 and 5 G is undoubtedly due to the descent of the diaphragm consequent to the increased weight of the abdominal contents as the level of the force environment increases.

The fact that the pericardial fluid behaves as a hydrostatic system also provides what is in effect a nearly perfect G-suit for the heart (2). In contrast, the lungs are very susceptible to acceleration. This is due to the large differences in specific gravity of the respiratory gases and blood and the resulting large change in ventilation/perfusion ratios which are exaggerated proportionally to the level of acceleration and the dimensions of the lungs (3).

In any event, due to the increased weight of the blood, maintenance of normal arterial pressure at heart level is not sufficient to compensate for the increased hydrostatic distance up to the brain during an exposure to acceleration (Figures 3 and 5). Consequently, a dramatic decrease in arterial pressure at head level occurs at the onset of the exposure, followed by a striking increase in pressure to hypertensive levels at heart level which is usually sufficient to restore circulation to the head at levels of acceleration of 4 to 5 G_z so that recovery of vision occurs if the acceleration is

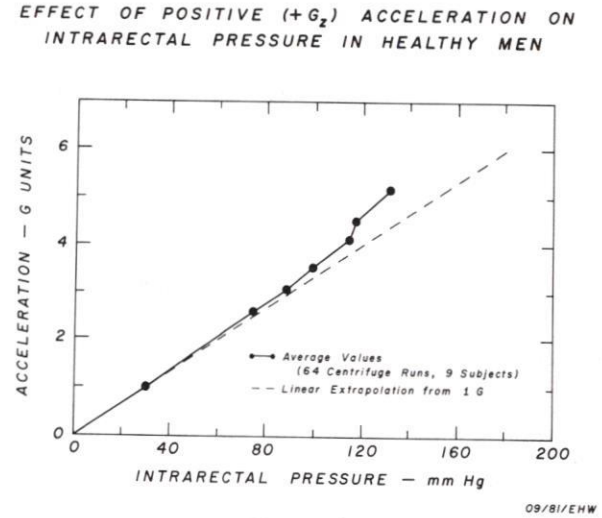


Figure 4

maintained for periods longer than about 7 seconds. That this compensatory hypertensive reaction is reflexly mediated can be demonstrated by autonomic blockade by tetraethyl amonium chloride as illustrated in the central panel of Figure 5 (4).

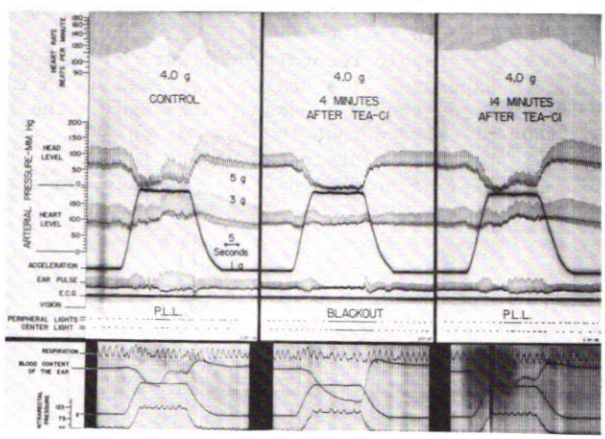


Figure 5

Activation of these protective baroreceptor reflexes prior to exposures to high levels of acceleration can be used to increase G tolerance. This can be done by an approximately 5 second exposure to a low level of acceleration prior to a high G exposure or by a gradual onset of acceleration to allow time for development of a compensatory hypertension at heart level before higher levels of acceleration are attained (5).

Another possibility is to use the Valsalva maneuver to induce a reflex hypertension prior to acceleration. The central panel of Figure 6 illustrates the hypertension that occurs following a 15 second duration Valsalva maneuver at 1 G and the right panel that this reflex hypertension can maintain clear vision at 4.0 G as contrasted to the blackout which this subject experienced during the prior control exposure, left panel.

The average subjective protection against visual symptoms in 21 subjects provided by this so-called M-2 maneuver of 1.3 G is supported by

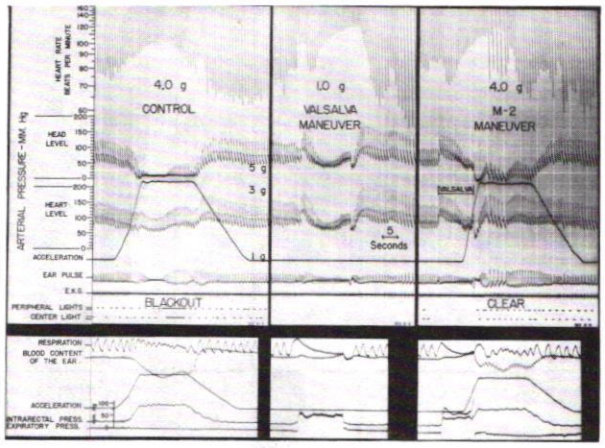


Figure 6

objective simultaneous photoelectric measurements of the blood content of the ear. The average protection afforded this index of circulation to the head in these same subjects ranged from 2.0 to 1.0 G. The highest value occurring at the onset of acceleration when the hypertensive reaction to the prior Valsalva maneuver was at its maximum.

A more effective means of protection against positive acceleration is to use the voluntary respiratory musculature to supplement the force of contraction of the ventricles.

An increase in airway pressure of 60 mm Hg (as illustrated in the center panel of Figure 7 produced by a forced expiration against a closed manometric system produces simultaneous increases in intra-abdominal and intraplueral pressure (6). The increase in pressure surrounding the heart and central vasculature is transmitted to the

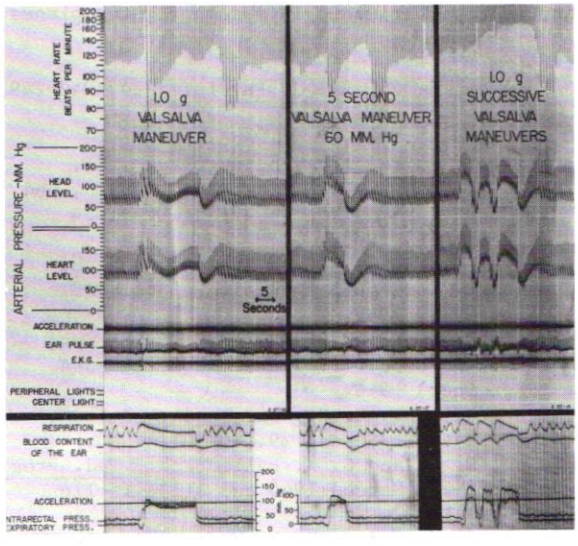


Figure 7

peripheral systemic arterial system producing a concomitant increase in systolic and diastolic pressure of about 50 mm Hg.

The subsequent decrease in arterial pressure due to decreased venous return, as illustrated in the left panel, can be abbreviated by rapidly exhaling and inhaling through an open airway followed as quickly as possible by a second 5 second period of increased airway pressure. As illustrated in the right panel, successive approximately 5 second duration hypertensive periods can be produced in this manner, separated by the brief hypotensive periods associated with each forced respiratory cycle.

An example of the protection against positive acceleration produced by this manuever is illustrated in Figure 8.

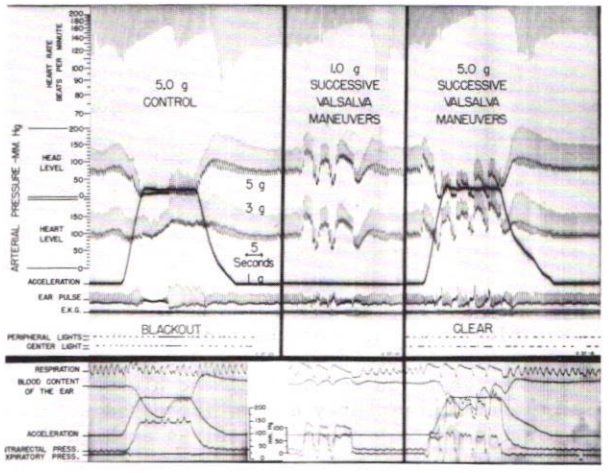


Figure 8

A decrease in the duration of the successive, undesirable hypotensive periods associated with each forced expiratory-inspiratory cycle during the maneuver can be accomplished by exhaling air slowly during the periods of increased airway pressure so that the lungs are near minimal residual volume after 3-5 seconds of the forced expiration (Figure 9, center panel). In this circumstance, only a rapid inhalation is required to reinitiate a period of increased airway pressure.

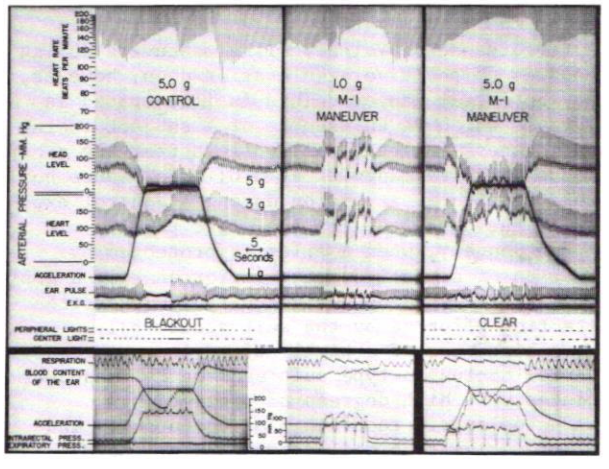


Figure 9

This is the so-called M-1 maneuver (7). An example of its effectiveness as a voluntary self-protective maneuver against the cerebral ischemia

produced by positive acceleration is illustrated in Figure 9.

This manuever was taught to a series of 28 healthy subjects back in the 1940s. Following oral and written instructions and coaching prior to centrifuge exposures, an average increase in G tolerance of 2 G was obtained. Written instructions only provided an average protection of 1.4 G - while coaching and a second trial on the centrifuge increased the average level of protection to 2.3 G (Table 1).

VARIATION IN THE PROTECTION AFFORDED BY THE M-1 MANEUVER WITH INSTRUCTION AND EXPERIENCE

Oral instruction and coaching		Written instruction only		Oral instruction and coaching with two series of centrifuge trials		
Sub-jects	Average protection*	Sub-jects	Average protection*	Sub-jects	Average protection* First series	Second series
35	2.0 (0.2-3.9)	6	1.4 (0.5-2.5)	5	1.6 (1.0-2.2)	2.3 (1.9-2.8)

*Average protection in g units afforded the ear opacity (blood content of the ear). Figures in parentheses are extreme values.

Table 1

Recordings of the blood content of the ear showed an average protection of 1.5 G at the lowest G tested as compared to 2.5 G at the highest level of acceleration, probably due to increased effort by the subjects to protect themselves at high G levels (Table 2).

VARIATION IN THE PROTECTION AFFORDED BY THE M-1 MANEUVER WITH THE MAGNITUDE OF THE ACCELERATION

	Protection in g units measured at:			
	Lowest g tested		Highest g tested	
Subjects	Average g	Average protection*	Average g	Average protection*
28	4.6 (3.0-7.0)	1.5 (0.2-2.7)	6.3 (4.0-9.0)	2.5 (0.1-5.1)

*Average protection afforded the ear opacity (blood content of the ear). Figures in parentheses are extreme values.

Table 2

Performance of Valsalva-like maneuvers during exposures to positive acceleration does, however, carry potential danger. This is illustrated in Figure 10 which is an example of a subject losing consciousness (right panel) while performing a continuous Valsalva maneuver at 3 G; an exposure he tolerated with clear vision during a control exposure to the same acceleration (left panel).

Fortunately, when a pilot is protected by inflation of an anti-G suit, performance of a continuous Valsalva maneuver actually increases the protection afforded by the suit alone (8).

Combined use of a highly effective progressive arterial occlusion type suit and the M-1 maneuver produces very high degrees of protection.

Photoelectric recordings of ear opacity made in 1943 during combined performance of the M-1 maneuver and inflation of the suit, indicate that the blood content of the ear can actually be increased above the 1 G level throughout a 12 second exposure to 9 G.

Colored pictures of subjects during such exposures document tremendous engorgement of the skin progressing downwards from ear level. This has

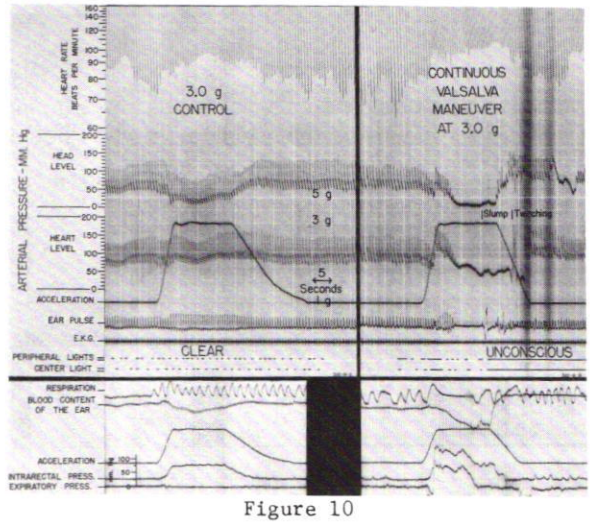


Figure 10

occurred to the degree that all non-pressurized areas of the skin below neck level were covered with petechial hemorrhages.

The danger of more serious sequelae from utilization of these high degrees of protection has not been clearly defined. Structural damage to the anatomically fragile lungs seems most likely due to the very high pressure gradients which would be generated at these high levels of acceleration between the zero specific gravity respiratory gases in the airway system and the mobile blood in the vasculature (9-11).

The M-1 maneuver or variants thereof are taught routinely to contemporary pilots of combat aircraft. This maneuver in combination with currently used G suits apparently provides effective protection at the very high levels of acceleration which can be experienced in modern fighter planes.

REFERENCES

1. Rushmer, R. F. Am. J. Physiol. 147:242-249, 1946.
2. Avasthey, P. and E. H. Wood. J. Appl. Physiol. 37(2):166-175 (August), 1974.
3. Wood, E. H., A. C. Nolan, D. E. Donald, and L. Cronin. Fed. Proc. 22(4):1024-1034 July-August) 1963.
4. Brown, G. E., Jr., E. H. Wood, and E. H. Lambert. J. Appl. Physiol. 2(3):117-132 (September) 1949.
5. Wood, E. H., E. H. Lambert, E. J. Baldes, and C. F. Code. Fed. Proc. 3(5):327-344 (September) 1946.
6. Bjurstedt, H., E. H. Wood, and A. Astrom. Acta Physiol. Scand. 29:190-201, 1953.
7. Wood, E. H. and G. A. Hallenbeck. Fed. Proc. 4(1):78-79 (March) 1945 and 5(1):115-116 (March) 1946.
8. Wood, E. H. and E. H. Lambert. J. Avia. Med. 23:218-228 (June), 1952.
9. Wood, E. H. Aerospace Med. 38(3):225-233 (March) 1967.
10. Sass, D. J., E. L. Ritman, P. E. Caskey, N. Banchero, and E. H. Wood. J. Appl. Physiol. 32(4):451-455 (April) 1972.
11. Craig, P. H., K. R. Coburn, R. F. Gray, and E. L. Beckman. Aerospace Med. 31:301, 1960.

GRAVITY, AND METABOLIC SCALE EFFECTS IN MAMMALS

Nello Pace and Arthur H. Smith

University of California, Berkeley 94720, and Davis 95616

In the evolution of life on Earth the mammals represent a form that began to emerge during the Triassic Period about 200,000,000 years ago. From then until now the group has shared a common set of distinguishing morphological and physiological characteristics. However, for the first 140,000,000 years of their existence the archaic mammals were small, ranging in body size from that of the modern shrew to modern rat. It was not until the early Tertiary Period, almost 60,000,000 years ago, that mammals rapidly began to develop the wide diversity in species body size evident today. The reasons for the surge in large body size that occurred at that time are not clear (14).

At present, the homologous category of placental mammals, the Eutheria, comprises more than 900 genera and 4,000 species (23). In this constellation the mature body mass ranges from 0.002 kg for the tiny Etruscan shrew, *Suncus etruscus*, at the lowest end, to the African elephant, *Loxodonta africanus*, the largest of the living terrestrial mammals with a body mass up to 12,000 kg. The fossil record reveals that the extinct hornless rhinoceros, *Indotherium transouralicum*, estimated to have attained a body mass of 16,000 kg, was probably the largest of the terrestrial mammals. Finally, the aquatic blue whale, *Sibbaldus musculus*, has been recorded as reaching a body mass of 203,000 kg. Thus, the terrestrial mammals exhibit a body mass range of 10^7 and, with the aquatic mammals, a range of 10^8. *Homo sapiens* is seen as a mid-sized placental mammal in this scheme.

GRAVITATIONAL LOADING

Throughout evolution, Earth gravity has imposed a loading force on terrestrial organisms directly proportional to their mass, according to Newton's Law of Gravitation: $F = G \cdot m_1 \cdot m_2 \cdot d^{-2}$. It is evident, then, that the loading force on mammals on the dry-land surfaces of the Earth today also varies over a range of 10^7.

More than 300 years ago Galileo recognized that when the gravitational loading force exceeds the cohesive forces that determine the strength of materials, a limit is reached for the size attainable by living organisms or artificial constructions. Furthermore, he enunciated clearly the concept that the breaking strength of a material is a function of its cross-sectional area, whereas the loading force is a function of its volume, or mass. Hence, the attainment of large size is possible only by using stronger materials or by disproportionate thickening of the component parts. D'Arcy Thompson (22) coined the term Principle of Similitude for Galileo's concept, and it forms the basis for the procedures of dimensional analysis used extensively in engineering for defining scale effects.

SKELETAL SCALE EFFECTS

Even a cursory examination of the morphology of the load-bearing skeleton of small organisms compared with large organisms of homologous groups reveals scale effects, such as a disproportionate thickening of the limb bones in mammals as larger and larger species are considered. For a number of years it had been appreciated by biologists (22) that the mass of the skeleton makes up an increasing proportion of the total body mass (TBM) as body size increases, and in 1963 Heusner (7) applied Huxley's allometric equation, $Y = aX^b$, to the phenomenon with the result

$$\text{Skeletal Mass,kg} = 0.093(\text{TBM,kg})^{1.142}.$$

A 5 gram shrew can be computed to have a skeletal mass of only 5% of its total body mass, whereas 35% of the body mass of a 12,000 kg elephant is skeleton. The clearly hypergonic relationship is affirmation that the Principal of Similitude applies in biology as well as in engineering.

In contrast to terrestrial organisms, aquatic organisms are considerably less subject to loading by gravity because of their buoyancy. Galileo also recognized that the fractional skeletal mass of large marine mammals should be smaller than that of their dry-land counterparts, and that they could attain far larger body sizes. Smith and Pace (19) surveyed Japanese whaling records and were able to derive an allometric relationship for skeletal mass as a function of body size among whales as follows

$$\text{Skeletal Mass,kg} = 0.116(\text{TBM,kg})^{1.024}.$$

It is evident that the relationship is not nearly as hypergonic as among terrestrial mammals, so that the skeletal fraction even of the blue whale is only 15% of the total body mass.

SCALING OF METABOLIC RATE

Another kind of scale effect that may reflect the influence of gravitational loading is the allometric relationship between metabolic heat production rate (MR) and total body mass. In one of those coincidences that seem to typify scientific discovery, Kleiber (11) and Brody and Procter (3) in 1932 simultaneously described the relationship by the now well-known equation

$$\text{MR,kcal}\cdot\text{hr}^{-1} = 2.84(\text{TBM,kg})^{0.748}$$

which yields resting metabolic rate for adults of terrestrial mammalian species from the mouse to the elephant. However, the physiological significance of the 3/4 power relationship of metabolic rate to total body mass has remained obscure.

If the individual tissue metabolic properties and proportions of the mammalian body were the same from species to species, one could expect the

metabolic rate to be directly proportional to body size; i.e., proportional to the 1.0 power of total body mass. If this were the case, however, the average temperature of the body would also be expected to increase with increasing body size because heat exchange with the environment occurs through the body surface, which increases only as the 2/3 power of body mass of comparable shape.

Yet, mammalian species of all sizes exhibit a relatively constant and closely similar body temperature, the phenomenon of homoiothermy. This fact led the 19th century physiologists to postulate the so-called Surface Area Law, which states that metabolic heat production for animals of different size is constant when expressed per unit of body surface area.

However, it is evident from the analyses of Kleiber and Brody that metabolic rate among mammals is in fact proportional to the 3/4 power of body mass, and not the 2/3 power as demanded by the Surface Area Law. Furthermore, Kleiber (12) has pointed out that heat transfer across the body surface is actually governed by Fourier's Law of Heat Flow

$$\dot{Q} = \lambda \frac{A}{d} (T_i - T_o)$$

where $\dot{Q}$ is the quantity of heat flow per unit time, λ is the thermal conductivity coefficient, A is the surface area, and d is the thickness of the region across which the temperature differential $T_i - T_o$ exists. Thus, it is clear that heat loss from the body is not only directly proportional to the body surface area, but is also inversely proportional to the effective thickness of the body surface layer separating the body core from the thermal environment.

Kleiber assumed skin thickness to represent the isolating surface layer, and found that it scales as the 1/5 power of total body mass over the size range from the rat to the cow. Since body surface area scales as the 2/3 power of body mass, then the ratio A/d in Fourier's equation is proportional to $TBM^{2/3}/TBM^{1/5}$, or $TBM^{7/15}$. Thus, if the thermal conductivity coefficient is independent of body size, as seems likely, heat transfer through the body surface should be proportional to the 7/15 power, or approximately 1/2 power, rather than the 2/3 power of total body mass.

SCALING OF THERMAL CONDUCTANCE

Support for Kleiber's hypothesis is found in the work of three groups of investigators (2,8,15) who have independently determined thermal conductance across the total body surface in various species of mammals of different body size at ambient temperatures below the thermoneutral zone. They derived the following allometric relationships of the weight-specific thermal conductance, C, to total body mass from their data

$$C,cal \cdot g^{-1} \cdot hr^{-1} \cdot {}^{\circ}C^{-1} = 4.91(TBM,g)^{-0.505} \quad (8)$$
$$C,cal \cdot g^{-1} \cdot hr^{-1} \cdot {}^{\circ}C^{-1} = 3.69(TBM,g)^{-0.426} \quad (2)$$
$$C,cal \cdot g^{-1} \cdot hr^{-1} \cdot {}^{\circ}C^{-1} = 4.85(TBM,g)^{-0.486} \quad (15)$$

The results agree reasonably well, and with appropriate averaging and transformation, may be summarized by the relationship

$$\dot{H},kcal \cdot hr^{-1} \cdot {}^{\circ}C^{-1} = 0.172(TBM,kg)^{0.528}$$

where $\dot{H}$ is the heat lost across the total body surface of the animal per hour per °C temperature difference between body core temperature and ambient temperature. It is seen that total heat transfer rate through the body surface does indeed appear to be more closely proportional to the 1/2 power of total body mass than to the 2/3 power, confirming Kleiber's view.

In any case, we are left with the dilemma that the mammalian characteristic of a constant body temperature would seem to require scaling of metabolic heat production as the 1/2 power of body mass, whereas the energy required for motor activity and maintenance of posture against the force of gravity should scale as the 1st power of body mass. In actual fact it scales as the 3/4 power of body mass. Hemmingsen (6) addressed himself to the dilemma, and concluded that the 3/4 power relationship probably represents an evolutionary compromise between the conflicting demands of proportionality of metabolism to body mass and proportionality to surface functions. However, he left unexplained the precise physiological nature of the compromise involved.

METABOLIC INTENSITY

One approach that may be made to an explanation is by examining the characteristics of the scaling of metabolic intensity, or metabolic rate per unit of body mass, as a function of total body mass. From the Kleiber-Brody relationship it follows that

$$MI,kcal \cdot hr \cdot kgTBM = 2.84(TBM,kg)^{-0.252}.$$

In other words, the smaller the animal the greater is the metabolic activity of each unit of body mass, on the average. The effect is a substantial one as may be appreciated from the fact that the metabolic intensity of the human body is 4 times greater than that of the elephant, while the metabolic intensity of the mouse is 25 times greater than that of the elephant.

Several investigators (1,10,13) have explored this remarkable physiological phenomenon by *in vitro* measurements of the oxygen consumption rate of slices of the major, metabolically-active body tissues from mammalian species of different size from the mouse to the horse, and deriving the allometric relationship between individual tissue QO_2 values and total body mass. It was their premise that the tissue QO_2 values might scale as the -0.25 power of total body mass, thereby accounting for the -0.25 power relationship of total body metabolic intensity to total body mass. However, as shown in Table 1, they found that tissue QO_2 values actually scaled between the -0.064 power of total body mass for kidney and the -0.266 power for striated muscle. A weighted average of their results indicates that tissue metabolic intensity scales more closely to the -0.15 power of total body mass than to the -0.25 power.

Other investigators have examined tissue parameters related to tissue oxygen consumption rate in similar fashion. R.E. Smith (20) made mitochondrial counts in liver homogenate preparations from 4 species from the rat to the steer, and found the number of mitochondria per gram of liver to scale as the -0.099 power of total body mass. Drabkin (4) estimated body cytochrome *c* content for 5 species from the rat to the horse, and the allometric relationship derivable from his data reveals that

cytochrome *c* content per kilogram of total body mass scales as the -0.164 power of total body mass.

Table 1. Values of b in allometric equation $Y = a(TBM)^b$

Y	b	Reference
Kidney QO_2	-0.064	(13)
Brain QO_2	-0.069	(13)
Lung QO_2	-0.095	(13)
Heart QO_2	-0.115	(13)
Liver QO_2	-0.153	(10)
Diaphragm QO_2	-0.266	(1)
Mitochondria per gram liver	-0.099	(20)
Cytochrome *c* per gram body	-0.164	(4)

BODY HEMOGLOBIN AND MYOGLOBIN

Drabkin (4) also measured total body hemoglobin and myoglobin contents for the 5 species of mammals he examined. As shown in Table 2, the allometric relationship of total body hemoglobin to body size scales as the 0.985 power of total body mass. This result agrees remarkably well with the finding of Brody (3) that the blood volume of mammals scales as the 0.987 power of total body mass. Thus, the blood oxygen transport system of the body seems to be related isogonically to body size.

Table 2. Allometric constants for $Y = a(TBM,kg)^b$

Y	a	b	Ref.
Body Hemoglobin, g.	13.5	0.985	(4)
Blood Mass, g	50.7	0.987	(3)
Body Myoglobin, g	0.573	1.233	(4)
Body Muscle Mass, kg	0.253	1.050	(19)

In contrast, the allometric relationship of total body myoglobin content to body size mass is markedly hypergonic, the body myoglobin mass scaling as the 1.233 power of total body mass. The body muscle mass is also hypergonic, but scales only as the 1.050 power of total body mass (19). Therefore, it appears that the myoglobin content per unit mass of muscle must increase as a function of body size. A possible explanation may lie in the fact that myoglobin is localized in the red, or Type I, muscle fibers associated with antigravity function, and that the proportion of red to white muscle fibers may increase as a function of body size. In support of this explanation is the additional fact that muscle myoglobin content can change in proportion to chronic loading on the muscle. Hence, it is tempting to speculate that body myoglobin content may be diminished in weightlessness.

SUMMATION OF TISSUE QO_2 AND ORGAN SIZE

It seems clear that while the metabolic intensity of the individual body tissues declines as a function of species body size, the decline is not sufficiently great to account entirely for the -0.25 power scaling of total body metabolic intensity on total body mass. In 1961, Kleiber (11) reviewed the various hypotheses which had been advanced to that point to account for the difference in scaling between *in vivo* total body metabolic intensity and individual *in vitro* tissue metabolic intensity. He concluded that the difference must reside in the fact that regulation of metabolic rate *in vivo* involves modulation of tissue metabolic activity by centrally integrated nervous and endocrine activity, but did not go beyond this generalization.

In 1967 Holliday et al. (9) made the important suggestion that the decline in total body metabolic intensity as a function of species size might be the resultant of a combination of two factors -- the decline in metabolic intensity of the individual tissues, and the change in relative proportions of the body organs as animals become larger and larger. It had been recognized for some time that the proportion of the metabolically highly active organs, such as the liver, kidneys and brain, becomes progressively less, while the proportion of the less active musculoskeletal system becomes correspondingly greater as body size increases among mammals. Thus, one might well expect that overall body metabolic intensity should decline as a function of total body mass. Holliday et al., however, did not subject their hypothesis to rigorous quantitative test.

Recently, Pace et al. (16) measured body organ masses in a series of small laboratory mammals from the mouse to the rabbit under standardized conditions, and were able to derive allometric relationships to total body mass. In their series, the body viscera scaled as the 0.871 power of total body mass, whereas the musculoskeletal system scaled as the 1.055 power. Using these data and QO_2 values from the literature, an equation has now been developed which yields total body metabolic rate as a summation of the metabolic rates of the individual major organs of the body over the size range 0.02 kg to 5 kg. The development of the equation and derivation of the equation parameters are to be described in a forthcoming publication, but it is of interest to note here that the 0.75 power regression of metabolic rate on mature body size can be explained entirely by the combination of tissue QO_2 regression on total body mass and change in relative organ proportions, as suggested by Holliday et al.

CHANGES IN GRAVITATIONAL LOADING

In an extension of Hemmingsen's ideas, Kleiber in 1969 proposed that the 3/4 power relationship of metabolic rate to total body mass may represent an evolutionary compromise between the 1.0 power demands of gravitational loading on the organism and the 1/2 power requirements dictated by thermoregulation (12). From Kleiber's hypothesis it follows that a change in gravitational loading could alter the power relationship of metabolic rate to body mass. Thus, removal of gravitational loading, as occurs during the weightlessness of space flight, should tend to shift the scaling of metabolic rate on body size toward the 1/2 power. Increases in gravitational loading, such as by chronic centrifugation, should tend to shift the scaling toward the 1.0 power.

Indication of the influence of gravitational loading on metabolic energy requirements comes from several sources. Taylor et al. (21) have shown that the basal metabolic rate of humans is reduced by about 10% after 3 weeks of continuous bed rest.

Smith (18) has presented evidence that when gravitational loading was increased in 3 species of animals of different body size by exposure to various levels of chronic acceleration, the daily maintenance feed requirement was increased in direct proportion to the acceleration level. He derived a 0-g intercept value for each species, and treated the 3 intercept values allometrically as a function of total body mass to obtain the relationship

$$0\text{-}g \text{ Feed Requirement, kcal/24hr} = 126(\text{TBM, kg})^{0.43}$$

which suggests that the regression of metabolic rate on body mass in weightlessness should be close to the 1/2 power of body mass. The data of Hemmingsen (5) indicate that the metabolic rate of a series of unanesthetized rats ranging in body mass from 0.08 to 0.20 kg scaled as the 0.77 power of body mass, whereas during anesthesia the metabolic rate scaled as the 0.56 power, again close to the 1/2 power of body mass.

From these considerations it may be predicted that the removal of gravitational loading, as occurs during the weightlessness of space flight, should result in a shift of the scaling of metabolic rate on body size from the 3/4 power toward the 1/2 power of total body mass. Conversely, an increase in gravitational loading, such as by chronic centrifugation, should shift the scaling toward the 1.0 power of total body mass.

In the interests of subjecting this hypothesis to experimental test, we have developed a 4-species small-mammal model which yields the normal 3/4 power relationship of metabolic rate to body size with an accuracy of ± 5% (17). The model comprises 6 metabolically mature animals of each of the 4 species, hamster, rat, guinea pig and rabbit. We are at present subjecting the model to chronic centrifugation at levels up to 2.5 g to learn whether, indeed, the scaling of metabolic rate can be shifted from the 3/4 power toward the 1.0 power of total body mass as a result of increased gravitational loading. It is also proposed to test the model in a future spaceflight experiment in order to determine whether the scaling of metabolic rate on total body mass can be shifted toward the 1/2 power in weightlessness. The results of these studies should provide further illumination of the role of gravity as a biological shaping factor for Earth organisms.

ACKNOWLEDGMENT

NASA travel support is gratefully acknowledged.

REFERENCES

1. BERTALANFFY, L.von and W.J. PIROZYNSKI. Tissue respiration and body size. Science 113: 599-600, 1951.
2. BRADLEY, S.R. and D.R. DEAVERS. A re-examination of the relationship between thermal conductance and body weight in mammals. Comp. Biochem. Physiol. 65A: 465-476, 1980.
3. BRODY, S. Bioenergetics and Growth. New York: Reinhold, 1945.
4. DRABKIN, D.L. The distribution of the chromoproteins, hemoglobin, myoglobin, and cytochrome c, in the tissues of different species, and the relationship of the total content of each chromoprotein to body mass. J. Biol. Chem. 182: 317-333, 1950.
5. HEMMINGSEN, A.M. The standard metabolism of female rats before and after ovariectomy in experiments with and without narcosis. Skand. Arch. Physiol. 68: 137-159, 1934.
6. HEMMINGSEN, A.M. Energy metabolism as related to body size and respiratory surfaces, and its evolution. Rep. Steno Mem. Hosp. & Nord. Insulin Lab. 9/2: 1-110, 1960.
7. HEUSNER, A. Cited by C. Kayser and A. Heusner. Étude comparative du metabolisme énergefique dans la série animale. J. Physiol. Paris 56: 489-524, 1964.
8. HERREID, C.F. and B. KESSELL. Thermal conductance in birds and mammals. Comp. Biochem. Physiol. 21: 405-414, 1967.
9. HOLLIDAY, M.A., D. POTTER, A. JARRAH and S. BEARG. The relation of metabolic rate to body weight and organ size. Pediat. Res. 1: 185-195, 1967.
10. KLEIBER, M. Body size and metabolism of liver slices *in vitro*. Proc.Soc.Exp.Biol.Med. 48: 419-423, 1941.
11. KLEIBER, M. The Fire of Life. New York: Wiley, 1961.
12. KLEIBER, M. Further considerations of the relation between metabolic rate and body size. In: Energy Metabolism of Farm Animals. K.L. Blaxter, J. Kielanowski and G. Thorbek, editors. Newcastle upon Tyne, Oriel, 1969, p. 505-511.
13. KREBS, H.A. Body size and tissue respiration. Biochim. Biophys. Acta 4: 249-269, 1950.
14. LILLEGRAVEN, J.A., Z. KIELAN-JAWORSKA and W.A. CLEMENS, editors. Mesozoic Mammals. Berkeley: University of California, 1979.
15. McNAB, B.K. On estimating thermal conductance in endotherms. Physiol. Zool. 53: 145-156, 1980.
16. PACE, N., D.F. RAHLMANN and A.H. SMITH. Scale effects in the musculoskeletal system, viscera and skin of small terrestrial mammals. Physiologist 22(6): S51-S52, 1979.
17. PACE, N., D.F. RAHLMANN and A.H. SMITH. Scaling of metabolic rate in small laboratory mammals. Physiologist 23(6): S115-S116, 1980.
18. SMITH, A.H. The roles of body mass and gravity in determining the energy requirements of homoiotherms. In: COSPAR Life Sciences and Space Research XVI. R. Holmquist, ed. Oxford: Pergamon, 1978, p. 83-88.
19. SMITH, A.H. and N. PACE. Differential component and organ size relationship among whales. Environ. Physiol. 1: 122-136, 1971.
20. SMITH, R.E. Quantitative relations between liver mitochondria metabolism and total body weight in mammals. Ann. N.Y. Acad. Sci. 62: 403-422, 1956.
21. TAYLOR, H.L., A. HENSCHEL, J. BROZEK and A. KEYS. Effects of bed rest on cardiovascular function and work performance. J. Appl. Physiol. 2: 223-239, 1949.
22. THOMPSON, D.W. On Growth and Form. J. T. Bonner, editor. London: Cambridge Univ., 1961.
23. WALKER, E.P. Mammals of the World (2nd ed., revised by J.L. Paradiso). Baltimore: Johns Hopkins, 1968.

RESTRAINT OF ANIMALS IN SPACE RESEARCH

R. R. Burton, J. W. Burns, and A. H. Smith

Crew Technology Division, USAF School of Aerospace Medicine, Brooks AFB, Texas 78235, and Department of Animal Physiology, University of California at Davis, Davis, California 95616

Restraint of animals for orientation purposes in a space (0G) environment for research purposes is reviewed relative to types of restraint; restraint tolerance criteria; and animal training, adaptation, and selection for restraint. Potential problems regarding animal restraint as well as considerations about restraint in space are discussed. The restraint process in space research was concluded to be a critical issue.

The unique weightless environment of space requires that some form of restraint must be utilized in order for animals to maintain orientation. This requirement is in addition to the use of animal restraint in scientific studies at 1G (on earth) to collect data. Since this orientation type of restraint must be used (worn) by the animal at all times and since data obtained from animals in space are usually compared with data obtained from animals at 1G, without orientation restraint, the restraint system becomes a variable without controls and therefore must be as nonencumbering as possible. "Nonencumbering as possible" is the key to successful restraint, yet since it must always, to some degree, encumber, it is useful to understand the "limits" of orientation restraint.

TYPES OF ORIENTATION RESTRAINT

Types of restraint used at 1G to simulate weightlessness of space will not be considered in detail, i.e., immobilization, bedrest, isolation, drugs, surgery or other methods to produce "hypokinesis." It is obvious that restraint systems which produce quasi-weightless physiologic responses would not be particularly useful in a study in space to determine the effects of weightlessness. Also immobilization types of restraint are known to be extremely stressful, frequently culminating in death. These types of severe restraint systems cannot be successfully tolerated although many attempts have been made to improve their restraint tolerance, e.g., restraint training, genetic selection, exercise training, or drugs.

Active Restraint:

Chronic Acceleration:
The use of the centrifuge--developing an ambient chronic accelerative field--has been considered as a method of orientation in space by Sluka et al. (24). They found that 0.02G was required to orient fertile chicken eggs for hatching in space. Yuganov and Pavlov (26), using weightlessness environments induced by parabolic airplane flights, found that approximately 0.4G would be required to stimulate electrical activity in the thigh muscle of small dogs in space. Presumably this amount of G would be required in space to properly orient small dogs. Burton et al. (10), using counterweighting (unloading) techniques, reported that approximately 50% of the animal's (chicken) weight was required for voluntary orientation. Using these data, they suggested that a ventrally directed load of approximately 50% of the bird's body mass would be required for orientation purposes in space, i.e., 0.5G could provide this force.

Two problems exist relative to using chronic acceleration forces for orientation in space: (a) eliminates the weightless environments and (b) can have profound pathophysiologic effects on animals if the G force is of sufficient strength (8).

The former problem can be reckoned with using an approach (data analyses) developed by Smith (25). This method requires at least three data points, each at different G levels, and by mathematically extrapolating to 0G, weight effects can be determined. Developing a data point between 0G and 1G (e.g., 0.5G and extrapolating only 0.5 unit) makes this method probably quite accurate and rather attractive (Fig. 1).

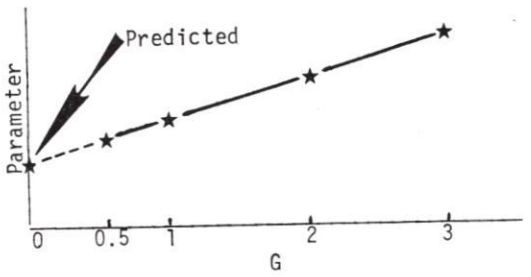


Fig. 1: The parameter is a function of G. Parameter at 0G is mass dependent.

The latter difficulty can be addressed by always using accelerative fields less than 1G. Pathologic effects from chronic accelerative exposures become apparent only at higher G levels and their incidence are directly correlated with the intensity of the accelerative field (8).

Passive Restraint:

Passive methods of restraint are specifically

directed towards types of animals being restrained. By using anatomical attributes peculiar to certain animals, passive restraint system can be optimized. For example, a perch can be used to great advantage in a restraint system designed for a chicken (9).

Since almost unlimited types of passive restraint systems exist or can be conceived, only examples of these systems will be considered.

Cages:
Cages which completely surround the animal, allowing for support in orientation without severe restraint penalties, are most useful in animals that can grip with their feet, i.e., rats, mice, domestic fowl, and primates. If animals cannot grip, such as rabbits, then restraining cages must be shaped to their "natural" posture (normal sitting) and although these are only lightly encumbering--described by the investigator as "light restraint"--they "almost invariably induced hypothermia" (14).

In most all studies at 1G (earth gravity), where cages (regardless of size) were used to restrain rats for physiologic studies, the cage per se produced restraint pathophysiologic effects--it has been shown that the degree of stress is inversely related to cage size (6). Even cages large enough for animals to change body positions 180°, such as laying cages for domestic fowl (hens), can produce a metabolic disease known as cage layer fatigue (osteoporosis) (13). It appears, therefore, that the use of cages is not a particularly good method for orientation restraint but quite the opposite; serves as an excellent stressor for studies of physiologic/psychologic stress.

"Chairs" and Form-Fitting Couches:
Non-human primates may be restrained in chairs or form-fitting couches. Quadrupeds and avian bipeds are not adaptable to "chairs" but can be restrained in form-fitting couches.

Immature, clinically healthy chimpanzees which had been restraint trained for several months were restrained supine in a form-fitting couch for 24 hours. Significant fluctuations in physiologic values, particularly in the hematropic and serum biochemical values, were found during the restraint period prompting the author to warn that "knowledge of restraint and its side effects" are necessary for successful space research (11). Berendt and Williams (2) found both "chair" restraint and position were important variables in respiration physiology studies in rhesus monkeys. Other types of chair restraint systems for non-human primates have been studied (15, 18) and all have been successfully tolerated, although Bouyer et al. (4) found significant changes in the electrocorticogram (ECoG) of chair restrained baboons which he considered to be signs of stress. This stress was relieved by presenting the animal with a task for his curiosity and hunger drive or by the administration of diazepam, an anxiolytic drug. Interestingly, chronic restraint of a primate in space (Bonny) was accomplished using a chair system.

Burns (7), in our laboratory, has developed a form-fitting couch for miniature swine in which their legs were extended through the bottom of the couch so that the animal was oriented in a prone position. While in this restraint system and left undisturbed for several hours, animals were found to be relaxed--some went to sleep. A study of six of these animals found extremely low plasma levels of norepinephrine (1.2±0.4 ng/ml), epinephrine (0.09±0.2 ng/ml), and cortisol (11.2±2.1 ug%, 4 animals) indicating that these animals were not stressed. This restraint system has been successfully used in accelerative research where animals were exposed to high $+G_z$ levels in cardiovascular studies (7). Animals were purposely stressed in these studies (n=7) and had norepinephrine, epinephrine, and cortisol plasma levels of 53.8 ng/ml, 58.0 ng/ml, and 34 ug%, respectively. A similar restraint system called the "Bird Box" was developed by Besch et al. (3) for adult domestic fowl. Their system probably encumbered the bird more than the Burns' system immobilized the pig. Unfortunately, the Bird Box produced 100% mortality on five birds with a mean survival time of 7.4±1.6 days. Other birds deprived of feed had a survival time of 12.8±0.4 days, thus indicating that the restraint birds' deaths were the result of a very active stressful process. Clearly, form-fitting couches are not useful restraint systems for all species of animals.

Harness-Sling; Tether; Collar:
Several types of these passive restraint systems have been developed and used with different species of animals. McEwen (19) used a Plexiglas collar supported on a pillar attached to a Plexiglas plate upon which a rabbit sat "restrained" with its head through the collar. Jensen et al. (16) restrained adult swine individually with a tether-collar arrangement. A harness-sling arrangement was developed by Besch et al. (3) for the domestic fowl and by Knize et al. (17) for the rabbit. Taping four feet "to uprights placed on the circumference of a 9 1/2 inch circle" was used by Bartlett (1) to "tether" guinea pigs.

These forms of passive restraint are similar to active restraint since the animal restrained is capable of maintaining normal posture, i.e., generally the restraint system is designed to support as well as control the animal in its normal postures. A "chair" has been designed for pregnant baboons which allows considerable physical freedom, i.e., in that regard it functions more like a harness than a chair (12). This chair-harness type of restraint was well tolerated by pregnant baboons, without interruption of gestation, for up to 107 days.

Animal tolerance to these types of passive restraints appears to be excellent although Besch et al. (3) found that their harness system caused death in adult domestic fowl. Later, Burton and Beljan (9) modified the Besch harness-sling restraint technique, allowing more animal mobility, and with some animal selection and training found it well tolerated by chickens. The

harness-sling technique worked well for restraining 36 rabbits for 4 days each--the rabbit is known for its high susceptibility to restraint stress (17, 19). The tethered restraint system used on guinea pigs was less stressful than caged restraint (1) and tethered female swine were not physiologically affected as determined by reproductive parameters (16).

RESTRAINT TOLERANCE CRITERIA

Systems available to determine animal tolerance to restraint are usually based on criteria used to measure the occurrence and degree of restraint stress or lack of restraint tolerance. Restraint, untolerated, elicits in animals the general stress syndrome described by Selye (22). Since this stress response involves nearly all of the physiologic and biochemic activities of the body, directly or indirectly, countless criteria are available to measure restraint intolerance.

The principal criteria--minimum restraint test--should be specific for each study relating to the goals of the research, e.g., if the investigation was concerned with the effects of space on specific parameters, then the animal's response to the restraint system must not involve either directly or indirectly those parameters.

Measurements of restraint tolerance that are considered specific for identifying physiologic stress include changes in: (a) levels of circulating "stress" hormones; (b) size or weight of "stress" organs and their hormone concentration; (c) levels of certain types of circulating blood cells; and (d) levels of metabolites of stress-produced biochemical products usually found in the urine.

Tolerance parameters less specific for the stress syndrome but which can be used as indicators of a generalized "stressed and unhealthy" condition include changes in: (a) body mass; (b) food and water intake; (c) thermal control responses; (d) body composition; (e) reproductive capacity and status; (f) neurophysiologic functions; and (g) identification of pathologies during necropsy.

The identification of an animal which is restraint stressed may be as simple as observing its gross behavior--stressed adult domestic fowl can be grossly identified at times by their characteristic posture (9)--or as difficult as carefully following several physiologic parameters in restrained fowl on a weekly basis and identifying only an occasional significant difference from control values (21).

An animal stressed by chronic applications of restraint adapts to the stressed state with functional and structural changes and return to a quasi-normal physiologic status. Using levels (%) of circulating lymphocytes as a criterion of restraint stress, Burton and Beljan (9) followed the adaptive process of domestic fowl. They identified three groups of birds: (a) never stressed by the restraint process; (b) stressed but recovered; and (c) remained stressed for the entire length of the study (22 days). Transiently stressed birds upon return to non-stressed status were found to be not significantly different from nonrestrained birds using several cardiovascular, respiratory, metabolic and hematologic parameters (9). However, the effect of the continuing dynamic process of adaptation to chronic restraint could have a significant impact--particularly on their physiological reserve (20). Since physiologic adaptation is a dynamic process, restrained animals must be continually monitored during the experiment to identify those animals that lose their restraint adaptation and become physiologically stressed (10, 21).

TRAINING, ADAPTATION, AND SELECTION

Besch et al. (3) advised persons involved with the restraint problem, with obvious signs of resignation and frustration, that "through training and genetic selection...a restraint adapted domestic fowl may be developed." That laboratory continued investigating restraint stress in the domestic fowl using restraint procedures modified from their early harness-sling system in addition to physiologic adaptation techniques through restraint training (9, 10, 21). Genetic selection procedures to develop a restraint tolerant breed of chicken were never attempted. However, Sines (23) was successful in developing strains of rats that were either more or less susceptible to restraint-induced ulcers. Francis (13) found strain differences in the incidence of cage layer fatigue--a form of restraint stress. Also species differences exist regarding restraint tolerance (incidence of gastric ulcers) suggesting a genetic basis for restraint susceptibility (5). It is emphasized, however, that no animal (including man) exists that is not susceptible to some form of restraint.

Physiologic adaptation to chronic restraint using training techniques has been successfully attempted by several laboratories. Two variations in adaptation approaches have been tried: (a) gradually introducing the animal to restraint by periodically increasing the duration of restraint and/or the degree of restraint (3, 18, 19), or (b) abruptly restraining the animal and monitoring the physiologic condition until death or adaptation to the system has occurred and the animal is no longer stressed--initially this latter restraint approach generally produces physiologically stressed animals after some finite period of time (9). Training (the period of adaptation) is extremely important, because it not only increases tolerance to light restraint (orientation type) systems, but it "establishes the fact that tolerance has occurred."

RESTRAINT WITH "UNLIMITED" FREEDOM: THE PARADOX

A weightless orientation system requires some form of orientation assistance, yet any form of restraint, regardless of how minimal, probably elicits some physiologic response. Frequently this response is debilitating--adversely impacting the results of the experiment. Considerations

regarding restraint in space follow:

(1) Selection of the type (species) of animal must depend upon the purpose of the experiment as well as the type of orientation restraint. It is not unlikely that the animal choice for the research objectives at 1G might not be the optimal choice in space because of the orientation restraint system requirements.

(2) Selection of the restraint system for orientation should always stress animal freedom for orientation postures. This freedom is considered by most restraint investigators as crucial. In an attempt to reduce the complexity of the entire restraint system and its encumbrance, the restraint for animal orientation should be integrated as much as possible with the restraint system for collecting data.

(3) Training, or rather exposing the animal to the restraint system until it has adapted, is most important. This validates the restraint system.

(4) Use of specific criteria to evaluate the adaptive condition of the animal relative to the restraint system is critical to establish that the animal is not stressed or that the condition of the animal will not affect the objective of the research. Adaptation evaluations must be conducted routinely during the course of the study.

The need for a thorough examination of the restraint process in space research is a critical issue. No valid controls can be established for weightless restraint systems except in the weightless environment and here the effects of space are pervasive and to a great extent unknown. Only diligence in experimental design with emphasis on the orientation restraint system will assure the scientific community that data from the space environment are not those from the restraint-stress environment.

1. Bartlett, R. G., Jr. 1959. Effects of restraint on oxygen consumption of the cold exposed guinea pig. J. Appl. Physiol. 14:46-48.

2. Berendt, R. F. and T. D. Williams. 1971. The effect of restraint and position upon selected respiratory parameters of two species Macaca. Lab. Ani. Sci. 21:502-509.

3. Besch, E. L., A. H. Smith, R. R. Burton, and S. J. Sluka. 1967. Physiological limitations of animal restraint. Aerosp. Med. 38:1130-1134.

4. Bouyer, J. J., L. Dedet, O. Debray, and A. Rougenl. 1978. Restraint in primate chair may cause unusual behavior in baboons; Electrocorticographic correlates and corrective effects of diazepam. Electroenceph. Clin. Neurophysiol. 44:562-567.

5. Brodie, D. A. and H. M. Hanson. 1960. A study of the factors involved in the production of gastric ulcers by the restraint techhnique. Gastroent. 38:353-360.

6. Brodie, D. A. 1962. Ulceration of the stomach produced by restraint in rats. Gastroent. 43:107-109.

7. Burns, J. W., M. H. Laughlin, W. M. Witt, J. T. Young, and J. P. Ellis, Jr. 1981. Pathophysiologic effects of acceleration stress in miniature swine. Aviat. Space Environ. Med. In press.

8. Burton, R. R. 1965. Chronic acceleration sickness. Aerosp. Med. 36:39-44.

9. Burton, R. R. and J. R. Beljan. 1970. Animal restraint: Application in space (weightless) environment. Aerosp. Med. 41:1060-1065.

10. Burton, R. R., A. H. Smith, and J. R. Beljan. 1971. Effect of altered "weight" upon animal tolerance to restraint. Aerosp. Med. 42:1290-1293.

11. Caraway, B. L. 1966. Physiologic values of the normal miniature chimpanzee under restraint. USAF-SAM-TR 66-100.

12. Fleischman, A. R. and R. A. Chez. 1974. A chair for the chronic study of the pregnant baboon. J. Med. Prim. 3:259-264.

13. Francis, D. W. 1957. Strain differences in the incidence of cage layer fatigue. Poult. Sci. 36:181-183.

14. Grant, R. 1950. Emotional hypothermia in rabbits. Am. J. Physiol. 160:285-290.

15. Howard, W. H., J. W. Parcher, and D. R. Young. 1971. Primate restraint system for studies of metabolic responses during recumbency. Lab. Ani. Sci. 21:112-117.

16. Jensen, A. H., J. T. Yen, M. M. Gehring, D. H. Baker, D. E. Becker, and B. G. Harmon. 1970. Effects of space restriction and management on pre- and post-puberal responses of female swine. J. Ani. Sci. 31:745-750.

17. Knize, D. M., R. C. A. Weatherley-White, D. J. Geisterfer, and B. C. Paton. 1969. Restraint of rabbits during prolonged administration of intravenous fluids. Lab. Ani. Care 19:394-397.

18. Mason, J. W. 1972. Corticosteroid response to chair restraint in the monkey. Amer. J. Physiol. 222:1291-1294.

19. McEwen, G. N. 1975. Thermoregulatory responses of restrained versus unrestrained rabbits. Life Sci. 17:901-906.

20. Pace, N. 1959. Metabolic patterns in stressful environments. Am. Soc. Heat. Refrig. Air-Cond. Engin. Trans. 65th Annual Mtg. 65:101-114.

21. Ray, G. T., R. R. Burton, and J. R. Beljan. 1970. Parenteral administration of fluids as the sole source of hydration: Application in space (weightless) environment. 41:1166-1169.

22. Selye, H. 1950. Stress. Acta Inc., Montreal.

23. Sines, J. O. 1959. Selective breeding for development of stomach lesions following stress in the rat. J. Comp. Physiol. Psychol. 52:615-619.

24. Sluka, S. J., A. H. Smith, and E. L. Besch. 1966. Orientation in systems with asymmetric density distribution. Biophys. J. 6:175-188.

25. Smith, A. H. 1975. Principles of gravitational biology. Chap. 4 In: Foundations of Space Biology and Medicine. Vol II., Book I. NASA, Washington D. C.

26. Yuganov, Y. M. and G. I. Pavlov. 1968. Possible magnitude of artificial gravity based on electrical activity of the skeletal muscles. In: Medico-biological Studies During Weightlessness. NASA CR-1578.

ANIMAL MODELS FOR SIMULATING WEIGHTLESSNESS

E. Morey-Holton and T. J. Wronski

Biomedical Research Division
NASA-Ames Research Center
Moffett Field, California USA

Space flight experimentation of biological systems is extremely limited and very costly. To better define those biological systems which might be altered in a gravity-free environment, a variety of animal models for simulating weightlessness have been developed. Such systems provide information on potential perturbations during space flight, particularly as related to unloading and fluid shifts, and are a cost-effective means of critically defining those parameters to be measured during flight. A list of these models is found in Table 1. The most widely used model, to date, has been some form of immobilization. Although confinement in a small cage has produced interesting data, such data are not always in agreement with that obtained from space flight (1). Many of the other models have utilized larger animals but few data exist on animals, other than rats, exposed to weightlessness; thus, the applicability of these systems is unknown as data do not exist to validate these models. Water immersion and bed rest are much utilized models for human responses to weightlessness; space flight data appear to be intermediate between these models in terms of onset of fluid shifts. Transsection of nerves produces a flaccid paralysis and gives a more exaggerated response than that which is seen during flight. Although free fall in airplanes or aboard small rockets is an excellent simulation of space flight, the duration of such experiments is less than 5 minutes and the launch phase of such flights can produce perturbations which might override any true effects of reduced gravity for such a short free fall period. Since head-down bed rest in humans was shown by the Soviets to be a better simulator of weightlessness than horizontal bed rest, a similar weightlessness simulation in animals seemed necessary. NASA, in conjunction with the Gravitational Biology Consortium, developed a rat model to simulate on earth some aspects of the weightlessness alterations experienced in space, i.e. unloading and fluid shifts. At the time this model was evolving, space flight data from the Soviet Cosmos series was becoming available and could be used to validate ground-based model systems.

The first version of the head-down suspended rat (2) involved binding an orthopedic harness to the back of an animal and suspending the rat at about a 30° head-down angle which totally unloaded the rear limbs but allowed the front paws to be used for locomotion. The rat could move in a 360° arc. The animals had ad libitum access to food and water. The major problem with this system (Mod I) was that the harness had to be rebonded if the experimental period exceeded 14 days. For chronic studies, the model system was modified (Mod II) according to the suggestions of Novikov and Ilyin (3,4) and this version of the model is depicted in Figure 1. All versions of the rat model contain the same critical components, i.e. unloading of the rear limbs, use of the front limbs primarily in a pulling mode, and head-down tilt to cause a fluid shift similar to that encountered during exposure to weightlessness.

Table 1. MODELS SIMULATING WEIGHTLESSNESS

I. IMMOBILIZATION
- A. Partial Body
  1. Small Cage; Confinement
  2. Chair/Lounge
  3. Partial Body Cast
  4. Water Immersion
  5. Lower-Body Positive Pressure
  6. Bed Rest
- B. Total Body
  1. Body Mold
  2. Body Cast
  3. Water Immersion

II. PARALYSIS
- A. Nerve Transsection
- B. Spinal Transsection

III. FREE FALL AT 1 G
- A. Parabolic Flight in Aircraft
- B. Sounding Rockets

IV. HEAD-DOWN SUSPENSION

Although data exist from both the Soviet Cosmos series and animal models simulating weightlessness, this report will concentrate on a comparison of Cosmos data with the head-down simulation model. Those data readily available include weight gain vs food intake, skeletal changes, muscle alterations, and total mineral excretion. Since different versions of the model were used to obtain the data, and animals were of different ages, strains, and body mass, all values have been expressed as a percent change from the control value.

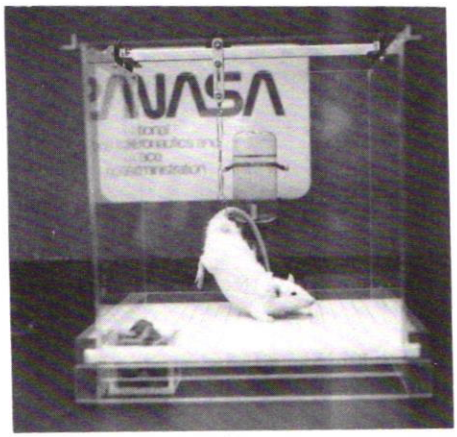


Figure 1. Head-down suspension model for simulating weightlessness in rats (Mod II).

WEIGHT GAIN AND FOOD CONSUMPTION

The initial comparisons between the head-down model and Cosmos data involved weight gain and food consumption (Table 2). If experimental animals received the same amount of food as the control groups (-1 to +4 % change from controls), the experimental animals gained considerably less weight; if, however, the experimental animals received 15-25% more food than the controls, their weight gain was similar. Thus, in all studies, flight and model, the experimental groups gained less weight per gram of food consumed than did the control rats. This consistent finding was unexpected, but may reflect loss of body water (5,6) or initial adaptation to a unique environment rather than an alteration in food assimilation.

Table 2. WEIGHT GAIN AND FOOD CONSUMPTION DATA FROM COSMOS AND HEAD-DOWN SUSPENSION

	WEIGHT GAIN (% Δ control)	FOOD CONSUMPTION (% Δ control)
COSMOS		
782	-61	0
936	- 3	+15
1129	-19	- 1
MODEL VERSION		
I	-53	0
I	- 6	+26
I	-59	+ 4
II	-28	+ 3

SKELETAL SYSTEM

Comparison of head-down suspension and space flight on skeletal parameters is found in Table 3.

Table 3. COMPARISON OF THE EFFECTS OF HEAD-DOWN SUSPENSION AND SPACE FLIGHT ON SKELETAL SYSTEM

PARAMETER	MODEL VERSION I/II	COSMOS 1129	COSMOS 936	COSMOS 782
	(% Change from Control)			
Bone Formation Rate (Tibiofibular junction)	-45/-35	-45	-45	-45
Trabecular Bone Volume				
Tibia (Proximal)	-65/ *	-35		
Humerus (Proximal)	-45/	-30		
Marrow Fat				
Tibia (Proximal)	+685/	+150		
Humerus (Proximal)	+500/	+270		
Endosteal Bone Resorption				
Tibia (Shaft)	0/0	0	0	0
Humerus (Shaft)	0/	0		
Osteoblast Population				
Humerus (Proximal)	-30/	-40		
Osteoclast Population				
Humerus (Proximal)	+90/	0		

*Blank spaces indicate the data do not presently exist.

Periosteal bone formation at the tibiofibular junction was suppressed approximately 45% in Cosmos 782, 936, and 1129 (7-9). The same measurements from Mod I also showed a 45% decrease in bone formation rate while Mod II had a 35% suppression of formation as compared with control animals; this slight difference might reflect the 2, rather than 3, week duration of the Mod II experiment as compared with the other experiments.

The trabecular bone volume was reduced similarly in the model and flight rats. Concomitant with the reduction in trabecular bone was an increase in marrow fat and decrease in osteoblast population in both the model and flight rats. The alteration in osteoblast population was only apparent immediately beneath the growth plate. The increase in osteoclast population in the model only, and the somewhat larger response in the majority of other parameters suggest an increase in glucocorticoids or "stress" response in this model. With the exception of the osteoclast data, the response in bone is strikingly similar between the model and flight animals.

SKELETAL MUSCLES

Comparison of head-down suspension and space flight on skeletal muscle parameters is found in Table 4.

Differential atrophy of skeletal muscles is apparent in both flight and head-down suspension (Table 4). The soleus muscles show greater atrophy than either the gastrocnemius or the extensor digitorum longus (EDL). Since the soleus supports posture, such atrophy during flight would be predicted. The data for differential muscle atrophy was obtained in our laboratory, but Musacchia and co-workers found similar results (12) in their modification of the model system. Maximum isometric tension was not significantly altered in the brachialis or EDL muscles either in the model or during space flight (1). However, decreased tension was found in the triceps brachii and the soleus in both systems (1).

Table 4. COMPARISON OF THE EFFECTS OF HEAD-DOWN SUSPENSION AND SPACE FLIGHT ON SKELETAL MUSCLES

PARAMETER	MODEL VERSION I/II	COSMOS 936	COSMOS 782
	(% Change from Control)		
Weight/100 g body mass			
Soleus	-45/-40	-35*	-20'
Gastrocnemius	-15/-20	-20*	0'
EDL	-10/- 5	-15*	
Maximum Isometric Tension (P_m)"			
Brachialis	/-15	+20	
EDL	/-20	+10	
Triceps Brachii	/-40	-30	
Soleus	/-55	-40	

Blank spaces indicate that data do not presently exist.
* Data from Ilyina-Kakuyeva and Portugalov (10)
' Data from Ilyina-Kakuyeva et al. (11)
" Data from Oganov et al. (1)

Skeletal muscle data obtained using head-down suspension strikingly resemble similar measurements obtained immediately following space flight.

TOTAL MINERAL EXCRETION

Comparison of head-down suspension and space flight on total mineral excretion is found in Table 5.

Table 5. COMPARISON OF THE EFFECTS OF HEAD-DOWN SUSPENSION AND SPACE FLIGHT ON TOTAL MINERAL EXCRETION

PARAMETER	MODEL	COSMOS 1129
	(% Change from Control)	
Calcium	-20 *	-20 '
Sodium	+135 **	+230 '
Potassium	↑ ***	+260. '

* Data from Musacchia et al. (13)
** Data from Musacchia & Deavers (14)
*** Parallels sodium (14)
' Data from Cann et al. (15)

Although the data from Cosmos for this parameter was measured on total excreta while the model data was collected separately for urine and feces, the changes in both systems are remarkably similar. Total excretion of calcium decreased by approximately 20% in both systems. Interestingly, the total body composition study on Cosmos 1129 indicated that body bone mineral (calcium) mass decreased by 20% (5). These data are difficult to correlate since food intake was similar in flight and flight control rats and since bone formation was suppressed with no change in bone resorption (15). Possibly the decreased excretion of calcium is reflecting a decrease in the size of the calcium pool during suspension and/or space flight.

The increased secretion of both sodium and potassium in both systems is puzzling. Similar observations were noted in the Skylab series in humans (16). Although increased aldosterone secretion was suggested as the reason for loss of potassium, this mechanism is not consistent with the loss of sodium. Musacchia has also noted the difficulty in explaining the increased potassium excretion paralleling the sodium excretion (14) and suggested that muscle atrophy may also partially responsible for the loss of potassium.

SUMMARY

Comparison of data collected from space flight and from the head-down suspension model for simulating weightlessness suggests that this model system reproduces many of the physiological alterations induced by space flight. Data from various versions of the rat model are virtually identical for the same parameters (Tables 2-4); thus, modifications of the model for acute, chronic, or metabolic studies do not alter the results as long as the critical components of the model are maintained, i.e. a cephalad shift of fluids and/or unloading of the rear limbs. Also, as long as young growing animals are used, alterations in strain, age, or weight do not appear to impact the conclusions.

Bone alterations in suspended and flight rats include a decrease in bone formation, a decrease in trabecular bone volume, an increase in marrow fat, and a decrease in osteoblast population. Bone resorption did not change in the bone shaft. Flight animals showed no indication of any alteration in bone resorption, whereas suspended rats did display an increase in osteoclast population in the metaphyseal region. However, this increase in osteoclasts may reflect a more severe response to a unique environment. The decrease in bone mass may contribute to the decrease in total body calcium. Whether the alterations in bone are due simply to unloading or whether they reflect other changes in fluid balance and/or calcium metabolism is unknown.

Skeletal muscle alterations in suspended and flight rats include a differential atrophy of the leg muscles with the soleus muscle being the most severely affected. The maximum isometric tension of the soleus and triceps brachii was decreased and, again, the soleus showed a greater suppression. Skeletal muscle atrophy may have contributed to the increased secretion of potassium. The postural or antigravity muscles lose more mass than do the other skeletal muscle groups.

Total mineral excretion in suspended and flight rats was very comparable. Calcium decreases and may reflect the decrease in skeletal mass since total body calcium is reduced similarly. Whether this calcium loss solely reflects alterations in bone structure or whether such a suppression of calcium indicates alterations in calcium homeostasis and other systems utilizing calcium has not been studied. The total body sodium is also decreased in flight rats and the total body potassium is suppressed, although total body potassium in flight rats is not

significantly different from controls (5). Both sodium and potassium excretion are significantly increased. These data are puzzling and suggest that either independent mechanisms are causing these alterations or unknown physiological control mechanisms are involved.

Thus, the head-down suspension model, when compared with available data from space flight, suggests that this model system is an excellent simulator of the alterations induced by space flight. Since data from the model and spaceflight compare so favorably, many of the effects of spaceflight might, indeed, be caused by fluid shifts and/or unloading of the musculoskeletal system. With this model system, one can now study the effects of unloading of the musculoskeletal system, independently or in combination with fluid shifts, on rat physiology. Although use of the model will allow more cost-effective definition of space flight experiments, the actual effects of weightlessness can only be studied in space. As Bjurstedt stated, "Although (this) ground-based simulation model must be fully exploited, essential aspects require sequential measurements with sophisticated techniques in the space environment. The results of such studies in man as well as in carefully selected animals will enhance our understanding of the physiology of health and disease on earth" (17).

REFERENCES

1. Oganov, W.V., S.A. Skuratova, A.N. Potapov, and M.S. Shirvinskaya. 1980. Physiological mechanisms of adaptation of rat skeletal muscles to weightlessness and similar functional requirements. The Physiologist 23:S16-S21.

2. Morey, Emily R. 1979. Spaceflight and bone turnover: Correlation with a new rat model of weightlessness. BioSci. 29:168-172.

3. Ilyin, E.A. and V.E. Novikov. 1980. A stand for simulation of physiological effects of weightlessness in laboratory experiments on rats. Space Biol. Med. 14(3):128-129.

4. Novikov, V.E. and E.A. Ilyin. 1981. Age-related reactions of rat bone to their unloading. Aviat. Space Environ. Med. 52:551-553.

5. Ushakov, A.S., T.A. Smirnova, G.C. Pitts, N. Pace, A.H. Smith, and D.F. Rahlmann. 1980. Body composition of rats flown aboard Cosmos 1129. The Physiologist 23:S41-S44.

6. Deavers, Daniel R., X.J. Musacchia, and Gerald A. Meininger. 1980. Model for antiorthostatic hypokinesia: head-down tilt effects on water and salt excretion. J. Appl. Physiol. 49:576-582.

7. Morey, E.R. and D.J. Baylink. 1978. Inhibition of bone formation during space flight. Science 201:1138-1141.

8. Holton, E. Morey, R.T. Turner, and D.J. Baylink. 1978. Quantitative analysis of selected bone parameters, pages 135-178. Final Reports of US Experiments Flown on the Soviet Satellite Cosmos 936. NASA Document TM-78536.

9. Wronski, T.J., E. Morey-Holton, and W.S.S. Jee. 1980. Cosmos 1129: spaceflight and bone changes. The Physiologist 23:S79-S82.

10. Ilyina-Kakuyeva, E.I. and V.V. Portugalov. 1979. Effect of artificial gravity on the rat skeletal muscles in spaceflight (aboard Cosmos 936). Arkhiv Anatomii, Gistologii i Embriologii 3:22-27 (Translated in NASA S&T Alert #5442).

11. Ilyina-Kakuyeva, E.I., N.V. Petrova, and V.V. Portugalov. 1979. The effect of space flight on the skeletal musculature and nerve apparatus of the muscles (morphologic and cytochemical study). Pages 100-106 in A.M. Genin, ed., The Effect of Dynamic Factors of Space Flight on Animal Organisms. NASA Translation Document TM-75692.

12. Steffen, J.M., X.J. Musacchia, and D.R. Deavers. 1981. Muscle atrophy and composition in the suspended hypokinetic rat: a model for weightlessness. Fed. Proc. 40:608.

13. Musacchia, X.J., Daniel R. Deavers, G.A. Meininger, and T.P. Davis. 1980. A model for hypokinesia: effects on muscle atrophy in the rat. J. Appl. Physiol. 48:479-486.

14. Musacchia, X.J. and D.R. Deavers. 1980. A new rat model for studies of hypokinesia and antiorthostasis. The Physiologist 23:S91-S92.

15. Cann, Christopher E., Richard R. Adachi, and Emily Morey-Holton. 1980. Bone resorption and calcium absorption in rats during spaceflight. The Physiologist 23:S83-S86.

16. Leach, Carolyn S. and Paul C. Rambaut. 1977. Biochemical responses of the Skylab crewmen: an overview. Pages 204-216 in R.S. Johnston and L.F. Dietlein, eds. Biomedical Results from Skylab. NASA Document SP-377, Washington, DC.

17. Bjurstedt, H. 1980. Remarks on present status of gravitational physiology. The Physiologist 23:S4-S6.

RESULTS OF INVESTIGATIONS OF WEIGHTLESSNESS EFFECTS DURING PROLONGED MANNED SPACE FLIGHTS ONBOARD SALYUT-6

O.P. Kozerenko, A.I. Grigoriev, A.D. Egorov

Institute of Biomedical Problems, USSR Ministry of Health, Moscow, USSR

The major result of the medical investigations in 96-through-185-day space missions onboard Salyut-6 was the well-documented demonstration of the fact that man can well adapt to such a long exposure to weightlessness and lead an active mode of life, performing sophisticated science and engineering experiments inside and outside the orbital station.

The physiological changes seen during and after flights were reversible and returned to normal after a relatively short period of readaptation; no linear correlation between the flight duration and the level of inflight and postflight reactions was found.

Summarizing the results obtained, it can be concluded that a prolonged exposure to weightlessness may induce homeostatic changes in different physiological systems, bringing the body to a different than on the Earth functioning level. The present communication discusses the data illustrating this notion.

According to the present-day concepts, the major factor responsible for the pathogenetic effects of weightlessness is the decrease of the weight-bearing loading on certain systems of the body due to the absence of weight and related mechanical tension of bodily structures (1-5).

In the weightless state, the functional unloading seems to be associated with changed afferentation from mechanoreceptors, fluid redistribution and diminished loading on the musculo-skeletal system. Subsequent involvement of a complex chain of reflex mechanisms appears to determine the resulting reactions, their pattern, level and sequence of development.

The crewmembers of the five long-term flights showed the most pronounced reactions immediately after insertion into orbit. They were sensory, vestibulo-autonomic disorders and symptoms associated with blood run to the head. Sensory disorders were related to illusionary sensations of inversion or displacement, which in cases were suppressed by gaze fixation, and of disproportionate muscle efforts involved in different actions.

Vestibulo-autonomic disorders, i.e., the state symptomatically similar to motion sickness (vertigo, loss of appetite, nausea, hypersalivation, and sometimes vomiting), were produced by rapid movements of the head and transit from a small into a large enclosure. These disorders were seen during the first 3 to 7 days with marked individual variations in terms of the level and duration. Sometimes vestibular discomfort (Commander of the 140-day flight) was observed inflight, and sometimes vestibular disorders were combined with statokinetic changes postflight.

Among the many concepts of the origin of space motion sickness, the theory relating vestibular changes to the dysfunction of sensors responsible for spatial orientation seems to be well-documented (6). It should be noted that the second exposure to weightlessness, as the Flight-Engineer of the 175- and 185-day flights remarked, "left a lesser impression, i.e., I did not have any specific sensations".

The cardiovascular system showed the most pronounced changes. The phenomenon of fluid redistribution, e.g., cephalad blood shifts, manifested beginning with the first hours of exposure to weightlessness as subjective dyscomfortable feelings (blood rush to the head, nasal congestion, head fullness, sometimes headache) and outward signs (facial edema, engorgement of neck veins, etc.).

Further cardiovascular shifts associated with a prolonged exposure to weightlessness were recorded as changes in central and regional circulation. According to rheographic data, they included an early (for about a week) increase in stroke volume and a slight increase in cardiac output as compared to the prelaunch values.

The pulse blood filling of cerebral vessels increased, returning to the normal as late as 3-4 months inflight (Fig. 1). This parameter in leg vessels remained diminished throughout the entire flight and in forearm vessels was either increased or unchanged.

According to occlusion plethysmography, venous pressure in legs decreased and in arms increased so that it was essentially at the same level. During the long duration flights the jugular vein pressure

was increased (Fig. 2). Thus, the pressure gradient in the upper and lower body was decreased. Leg venous distensibility increased and compliance decreased (7).

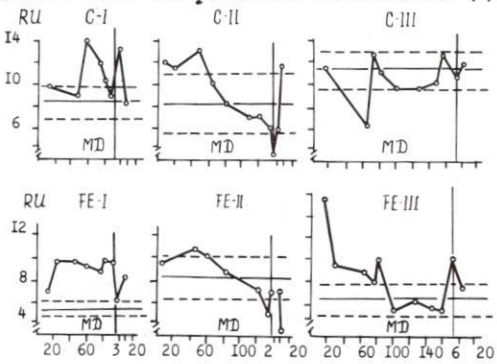


Fig. 1. Changes in pulse blood filling of the cerebral vessels in the 96-, 140- and 175-day Salyut-6-Soyuz crewmembers. MD=mission day. RU=relative units. Broken line - actual values at different stages of the orbital flight. Solid line - mean values preflight. Dashed line - limits of variations preflight. C-I, C-II, C-III - Commanders of the 96-, 140- and 175-day flights. FE-I, FE-II, FE-III - Flight-Engineers of the respective flights.

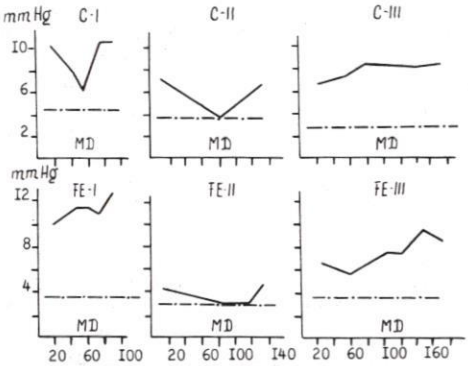


Fig. 2. Changes in venous pressure in 96-, 140- and 175-day Salyut-6-Soyuz crewmembers. Dashed-dot line - mean value preflight. Solid line - mean value inflight.

Changes in the myocardial bioelectric activity (positional shifts, ECG changes, T-wave depression) were of no clinical significance, did not influence circulation and recovered within a month postflight.

A prolonged exposure to weightlessness led in some cases (96- and 140-day flights) to cardiovascular deconditioning as indicated by exercise and LENP tests (8,9). In the 175- and 185-day crewmembers no cardiovascular deconditioning was seen and in the Flight-Engineer, who was exercising very heavily, the inflight reactions to the tests were lower than prelaunch. Cardiovascular deconditioning during exercise tests was very distinct immediately after landing and disappeared within an appreciably shorter time than after, say, the 30-day flight. After these long-term flights orthostatic tolerance was not less than after the 16-, 18- or 63-day flights; however, its recovery took longer time. This is undoubtedly the result of an adequate use of various countermeasures and a proper work-rest arrangement during Salyut-6 flights.

It is striking that postflight reactions to head-down tilts were inversely proportional to the flight time. It is very likely that an increased tone of upper body vessels and their decreased distensibility play a certain role in the mechanisms of adaptation to the weightlessness-induced blood redistribution.

Thus, stable cranial fluid redistribution in the weightless state, evidently, triggers mechanisms adjusting the circulation system to the new environment, the major changes being: decrease of the venous pressure gradient with the pressure reaching the level of the central venous or right-atrial pressure; increase in the active role of the diastole and subsequent development of the phase preload syndrome and involvement of unloading reflexes from the receptors of pulmonary veins; fluid removal according to the Gauer-Henry mechanism; increase of blood pools with partial compensation of the changes; involvement of compensatory mechanisms from the carotid sinus and stabilization of the new circulation level to provide an adequate supply of energy processes in the body.

As known, at an early stage of exposures to real and simulated weightlessness fluid-electrolyte shifts include increased renal excretion of extracellular fluid combined with inhibited water and salt requirements (5, 10). These phenomena are normally associated with the regulation of an increased effective blood volume due to the interstitial fluid release into plasma (Gauer-Henry reflex). After the 96-through-185-day flights changes in the fluid-electrolyte balance were: fluid retention despite increased water consumption, decreased sodium excretion and increased calcium and magnesium excretion in the urine with slight changes in the electrolyte concentration in blood. The loading tests demonstrated a mismatch in the system of ion regulation, opposite changes in the renal excretion of fluid and certain ions. The renal function did not alter significantly but the function of the concentrating system was disturbed and its reaction to the antidiuretic hormone was paradoxical. It was hypothesized (5) that changes in fluid-electrolyte balance may reflect weightlessness-induced adaptive rearrangements in the sensory, circulatory, and musculo-skeletal

systems as well as in involved mechanisms of neurohormonal regulation.

The negative potassium balance is very likely to be associated with atrophic muscle changes and muscle mass losses, therefore, with a total cellular potassium decrease; with respect to the regulation the negative potassium balance can be related to increased activity of insulin in blood which enhances tissue tolerance to free potassium and stimulates its urinary excretion.

Increased calcium excretion can be attributed to changes in bone metabolism in the weightless state. This is indicated by a decrease of the calcium concentration in skeletal bones. For instance, calcium losses from the heel bone after the 175-day flight were 3.2 - 8.3%. The mechanism of calcium turnover in weightlessness may involve an increased release of parathyroid hormone and thyrocalcitonin into blood.

It should be amphasized that all the above changes in fluid-electrolyte balance were reversible. Indirect data about inflight changes in water content were obtained through body mass and leg volume measurements. Beginning with the 140-day flight, body mass measurements were taken inflight. During the first flight days some crewmembers showed body mass losses and all of them displayed leg volume losses. This initial effect seems to be associated with fluid losses. The crewmembers of the 140- and 175-day flights (except for the Flight-Engineer of the 175-day flight) exhibited body mass losses, which were, as a maximum, 3.4 and 5.4 kg, and 4.4 kg, respectively. The Flight-Engineer of the 175- and 185-day flights and the Commander of the 185-day flight showed body mass gain which varied during the flight within the range of 4.3-4.7 kg and 1.9-3.2 kg, respectively, and stabilized after mission day 140 (Fig. 3).

The major body mass losses in the long-term flights are probably brought about by dehydration and muscle mass losses due to metabolic shifts induced by decreased weight-bearing loading. Of certain importance is increased physical and emotional activity (e.g., extravehicular activity), nutritional factor (selective drop-out of certain items from the diet), and other factors that can hardly be taken into account. Beginning with the first mission days, leg volume losses were seen in all cosmonauts (Fig. 4).

A prolonged exposure to weightlessness influenced the hemopoietic function and blood composition. Immediately postflight, the crewmembers of five long-duration space missions showed an increased content of hemoglobin, erythrocyte and leucocyte count (neutrophil leucocytosis and eosinopenia) and hematokrit. Later, these parameters progressively declined and reticulocytosis developed, and 1.5 months postflight they returned to the prelaunch level. A comparative analysis of the blood picture after flights of varying duration gave evidence that hemoglobin losses were proportional to the flight duration during the first 30 days only. After 96-, 140- and 175-day flights this parameter diminished on the average by 24%, 16% and 18% respectively. Thus, the hematological studies point to the inhibition of hemopoiesis during exposure to weightlessness and its activation upon return to Earth.

In summary, changes in certain functional parameters during and after long-term space flights are indicative of weightlessness-induced physiological shifts. They seem to be supported by the homeostatic mechanisms which make up for flight-related changes in vital constants, in order to provide a beneficial adaptive effect (nervous and humoral influences).

When discussing the results obtained in the long-term manned flights onboard Salyut-6, it should be borne in mind that the effects of weightlessness are closely associated with those of many other factors (confinement, isolation, monotony, diminished social contacts, etc.) constituting the space environment. With increase in flight duration their specific weight may vary, causing changes in the human body that may become very important as related to psychic adaptation. Extensive ground-based investigations of the effects of confinement and isolation on human psychics demonstrated specific responses. In particular, it was hypothesized that perceptive and intellectual functions may change due to sensoristasis, i.e., lowered stimulation by the specific environment that reduces the sensory diversity to which humans are accustomed (11, 12).

Our medical and psychological observations over the crewmembers who made five prolonged flights give evidence that they well tolerated the exposure, especially when psychoprophylactic measures and rational arrangement of work-rest cycles were provided. In addition, functional changes were seen that could be associated with psychoemotional changes. For instance, during mission days 28 through 49 the cosmonauts made frequent and insistent requests for musical, especially rhythmical, pieces to be relayed. In consistenoy with the activational theory of emotions (11), this can be described as a "stimulation-search behaviour" (13-14) or at least as an indication of changed functional requirements and needs for external stimulating reinforcement.

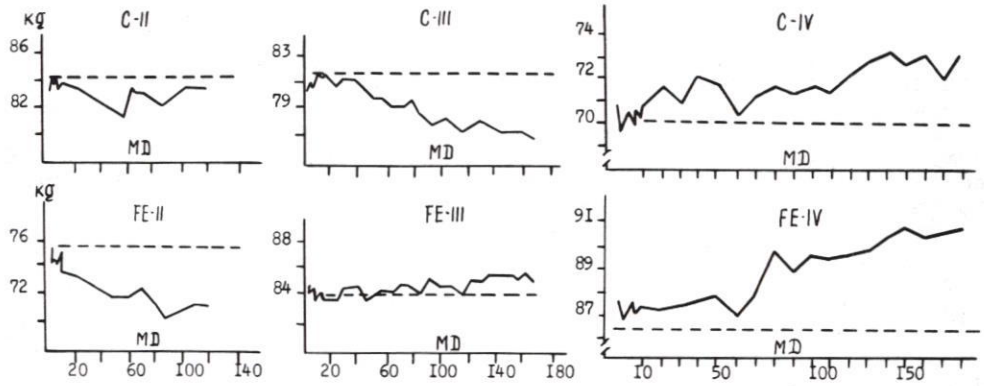


Fig. 3. Body mass of the Salyut-6-Soyuz 140-, 175- and 185-day crewmembers. Dashed line - mean value preflight. Broken line - mean value inflight.

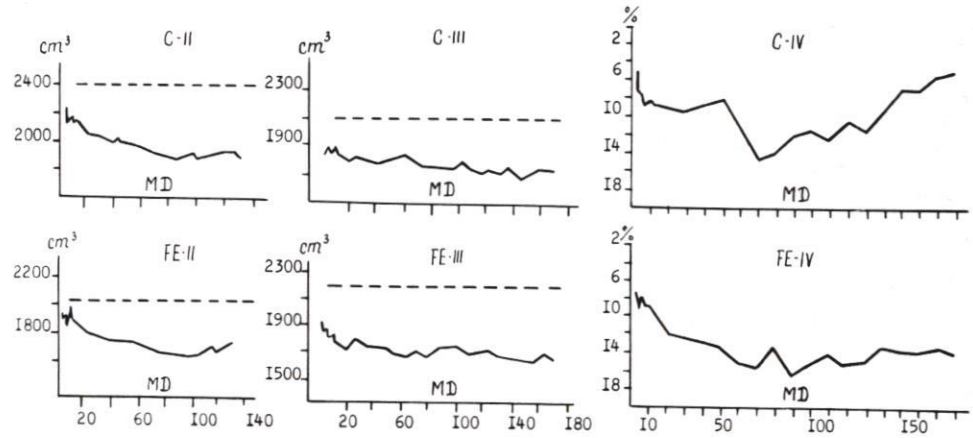


Fig. 4. Leg volume changes in the Salyut-6-Soyuz 140-(cm^3), 175-(cm^3) and 185-(%) day crewmembers.

Beginning with mission day 40, the 96-day crewmembers showed episodes of unstable mood, autonomic-somatic disorders and sleep disturbances. During that period they voiced complaints that they became tired not only by the end of the working day but also soon after awakening. After mission day 70 the few autonomic-somatic changes reached the level of moderately expressed asthenic manifestations.

The crewmembers of the 140-day flight exhibited more marked sleep disorders: in the Commander they were polymorphic and in the Flight-Engineer they were mostly associated with problems of falling into sleep.

Similar changes in the sleep pattern were seen in other cosmonauts, too. However, in the 175-day crewmembers they were visible in the lowest degree. It should be emphasized that these problems did not affect cosmonauts' work capacity. In our opinion, these changes should be considered in the context of mechanisms of continuous psychological adaptation to the environment. The study of the reactions as a function of flight time allows at least two stages to be distin-

guished in their dynamics. The first stage covers the early (for about four weeks) period of the long-duration space flight and depends on psychic rearrangement in the course of practical habituation to the environment and fulfillment of professional problems in this situation.

The second stage of psychological adaptation encompasses the remaining flight period and depends on predominant effects of psychological factors (isolation, monotony, deficiency of social contacts, etc.) enhanced by the effects of weightlessness.

In order to prevent potential negative effects of these factors on the psychic activity of cosmonauts during prolonged flights, many psychoprophylactic measures (psychological support) based on informational exposures were used (15). The crewmembers were supplied with emotionally and socially important information (new broadcasts, musical and television programs, talks with family members, famous representatives of arts and culture, scientific consultations, etc), as part of the general program of proper arrangement of their life and work (arrangement of leisure time, psychological reconstruction of the environment, motivational reinforcement of activities). We considered this to be a very important approach to the emotional-motivational regulation, involving the neuro-physiological apparatus of emotions. The use of such an approach in combination with other countermeasures have made, as we hope, an important contribution to the efficient adaptation of crewmembers throughout the long-term space flights.

In conclusion, it should be stressed again that all the behavioural and systemic changes, induced by space flight effects and specific environmental conditions, remained within the limits of adaptive reactions, thus indicating that the human body has wide reserves of adaptation to the life and work in the weightless state.

REFERENCES

1. Gurovsky, N.N. and Egorov, A.D. 1977. Some Problems in Space Medicine. Kosm.Biol.Aviakosm.Med. 11, 1, 3-13

2 Kovalenko, E.A. 1973 In: Pathological Physiology of Extremal States. Moscow, Medizina, 312-332.

3. Korshuev, P.A. 1971. Evolution, Gravity, Weightlessness. Moscow, Nauka, 152 pp.

4. Bjurstedt, H. 1979. Biology and Medicine in Space. Research Opportunities Offered by Spacelab. (ESA BK-01).

5. Gazenko, O.G., Grigoryev, A.J., Natochin Yu. V. 1980. Fluid-electrolyte homeostasis and weightlessness. Kosm.Biol.Aviakosm. Med. 14,5,3-10

6. Benson, A.J. 1977. Possible mechanisms of motion and space sickness. In: Proceed. European Symp. Life Sciences Res. in Space, Cologne Porz, Germany (ESA SP-130, 101-108).

7. Turchaninova, V.F. and Domrachiova, M.V. 1980. Study of the pulse blood filling and regional vascular tone during the first and second expeditions of the orbital station Salyut-6-Soyuz. Kosm.Biol.Aviakosm.Med. 14, 3, 11-14

8. Egorov, A.D., Itsekhovsky, O.G., Polyakova, A.P. et al. 1981. Study of hemodynamics and systolic time intervals of the 140-day crewmembers during exercise tests aboard Salyut-6. Kosm. Biol. Aviakosm. Med. 15, 3, 18-22

9. Degtyarev, V.A., Andriyako, L.Ya., Mikhailov, V.M. et al. 1980. Circulation reactions to the LBNP provocative test in the Salyut-6 first crew. Kosm.Biol. Aviakosm. Med. 14, 5, 29-32

10. Leach, C.S. 1979. A review of the consequences of fluid and electrolyte shifts in weightlessness. Acta Astronautica 6, 1123-1135

11. Lindsley, D.B. 1960. Attention, consciousness, sleep and wakefulness. In: Handbook of Physiology Neurophysiology, III. Y. Field. Ed., Am. Physiol.Soc., Washington, D.C, 1553-1599

12. Schultz, D.P. 1965. Sansory Restriction: Effects on Behaviour. Acad. Press. N.-J.

13. Scott T., Bexton W.H., Heron W., Doane B.K. 1959. Cognitive effects of perceptual isolation. Can.J. Psychol., 13, 200-209

14. Kuznetsov, O.N., Lebedev, V.I. 1972.
Psychology and psychopathology of Solitude.
Moscow, Nauka

15. Myasnikov, V.I., Kozerenko. O.P. 1981.
Prevention of psychoemotional disorders in prolonged space flights by methods of psychological support. Kosm. Biol. Aviakosm. Med. 15, 2, 25-29

RESULTS OF BIOSATELLITE STUDIES OF GRAVITY-DEPENDENT CHANGES IN THE MUSCULO-SKELETAL SYSTEM OF MAMMALS

V.S. Oganov

Institute of Biomedical Problems, USSR Ministry of Health
Moscow, USSR

The musculo-skeletal system as a tool providing locomotion emerged in the biological evolution of higher vertebrates simultaneously with their egress from an aquatic to a terrestrial environment. Further development and improvement of the musculo-skeletal system in different vertebrate classes proceeded under the influence of the Earth gravitational field.

The data available make it possible to reconstruct certain critical stages in the phylo- and ontogenesis of skeletal musculature of mammals (elastic structure of the musculo-skeletal system) associated in a different degree with gravity effects: the presence of tonic muscles in amphibians and their absence in mammals, dissimilar differentiation of fast and slow myofibers in lower vertebrates and mammals (8), specific features of the development of the contractile function in the pre- and postnatal ontogenesis of mammals, etc. (28). The data published in the literature also discuss, in general, the role of the gravitational field in the biochemical formation of phylogenetically young bones (rigid structure of the musculo-skeletal system) (11, 12, 20) and involvement of gravity in the development of locomotor and postural functions of extremities (5, 27).

In investigations onboard the biosatellites of the series Cosmos provided the material for experimental study and detailed analysis of changes in the musculo-skeletal apparatus of rats as a gravity-dependent system.

It was previously noted that one of the major results of Cosmos-flown experiments was the demonstration of the fact that the flight-induced changes in rat skeletal muscles and bones were more significant than those in other physiological systems, those former being defined as specific (4).

It was shown in particular that changes in the physiological properties of rat skeletal muscles were systemic and that their level depended on the degree of muscle involvement in the antigravitational function (17). The investigations of intact muscles (Cosmos-605, Cosmos-690) (16) and glycerinated muscle fibers (Cosmos-936, Cosmos-1129) (17, 23) demonstrated that the slow muscle of the hindlimb (soleus m.) and the extensor muscle of the forelimb (the medial head of the triceps brachii m.) showed a similar decline of the contraction force and work capacity. The contractile properties of the fast muscle of the hindlimb (extensor digitorum longus m.) changed insignificantly. The contraction force of the fast muscle of the forelimb (brachialis m.) either increased singificantly or remained unchanged. The contraction development was substantially accelerated in the soleus and slightly in the extensor digitorum longus muscles. Opposite changes were detected in the forelimb muscles examined. It was found that the effects seen in the forelimb muscles, including the hyperfunction of the brachialis m., were neutralized under the influence of artificial gravity (19) (hindlimb muscles of rats exposed to artificial gravity were not examined).

As shown by the investigations onboard Cosmos-1129, the changes in physiological characteristics of skeletal muscles at zero-g were accompanied and probably induced by alterations in the submolecular composition and physico-chemical properties of contractile and regulatory proteins (23).

The results of Cosmos rat experiments and the data in the literature allow the conclusion to be made that space flight may induce not only atrophic changes in the slow antigravitational muscles but also adaptive and biologically expedient transformations of phenotypes of muscle fibers, including, in all likelihood, reprogrammed synthesis of contractile muscle proteins. This may cause rearrangement of the functional profile of muscles: the slow antigravitational soleus muscle acquires the features typical of fast muscles, whereas the fast brachialis muscle gains those peculiar to slow muscles.

As follows from the comparative evaluation of flight and ground-based simulation studies (17), the major factor responsible for such reconstruction in space flight is a shift in the biomechanics of the contractile function of muscles to which they have adapted in the

course of evolution. For the antigravitational muscles this factor is mechanical unloading, while for flexor muscles of the forelimb (in rats) this factor is their involvement in an alien activity of the ronic type. The hyperfunction of the brachialis muscle could have been explained by the fact that in the support-free environment the flight animals might preferentially use highly developed forelimbs to stabilize their body and take meals (10). The biomechanical situation in the flight experiment suggests that flexor muscles, to which the brachialis m. belongs, are most actively involved in these locomotion tasks.

The major mechanism of the above-mentioned rearrangement may be an alteration of nervous-trophic effects initiated by the proprioreceptors, the effects controlling the structure and function of skeletal muscles (15).

The results of bone studies carried out in biosatellite rat experiments are also very significant, although they cannot be interpreted as unambiguously as the muscle data.

Of great importance is, in our opinion, the demonstration of the fact that in space flight the rate of periosteal osteogenesis and apposition growth decreased, and the rate of osteoid maturation and the degree of mineralization of tubular bones, tibial bones in particular, diminished (7, 14, 24). It can be speculated that osteogenesis of a compact bone may slow down to reach a complete arrest on mission days 11-12 (14). At the same time the biosatellite studies, as interpreted in (7, 14, 24), yielded no unequivocal information that may point to stimulation of bone resorption.

However, the biosatellite experiments supplied data indicating that the primary spongiosa in the proximal segments of tibia (2) and femurs (25, 26) diminished in mass and became loose. These findings can be accounted for by an enhanced resorption of the "perilacunar osteolysis" type (26).

This interpretation cannot be readily reconciled with the observations in (3) which give evidence that beginning with mission days 10-12 calcium excretion declines and by the end of the 18.5-day flight (Cosmos-1129) reaches the level 20 - 25% lower than that in the synchronous controls. The authors of the publication consider this phenomenon as a result of a total decrease of the rate of calcium turnover in calcified tissues, taking us back to the hypothesis of "a lazy bone" (13) used as a basis in the explanations of histological changes in bones of patients bed-ridden for a long time.

As much as the contractile properties of skeletal muscles represent their general status, biomechanical properties of bones are an integral indicator of the skeleton. In this context, there are interesting data indicating a decrease of mechanical strength of tubular bones (tibia and femur) by 20-30% (21, 22), reduction of tensile strength of vertebrae and an increase in the load sensitivity of the spinal column at the level of $T_9 - L_1$ (9). These changes in the biomechanics of tubular bones are normally attributed to the increased bone resorption, although the potential contribution of inhibited bone neoformation cannot be ruled out (22).

It is advisable to discuss the data presented in terms of the role of the postulated lack of the mechanical load on the skeleton in the weightless (support-free) environment as compared to the possible changes in other mechanisms (hormonal, nervous-trophic, vascular) regulating bone metabolism (13, 14).

We think that the term "mechanical load" needs clarification. As known, mechanical stimulation is mandatory to keep bones normally functioning (Wolf's principle) and bone osteogenesis and resorption adequately balanced (1, 6). However, in the Earth gravitational field this stimulation is determined by two components: mechanical (weight-bearing) load per ce and muscle forces acting upon skeletal bones during motor and postural activity. It is still disputable what is the specific contribution of each of the components into the regulation of bone metabolism (6). Along these lines another question can be raised: to what extent a change in either component is responsible for the mismatch of bone growth and remodelling which, as follows from the above discussion, may develop under the influence of space flight effects.

If we try to find an answer to the above question using the data available, which are obviously insufficient, we have to differentiate them as applied to different bone-muscle formations, for instance fore- and hindlimbs of rats, whose structural and functional differences are determined by the evolutionary and environmental changes.

The Cosmos-1129 experiment showed that the flight-induced inhibition of osteogenesis of the humerus was twice weaker than that of the tibia (24).

Previous studies onboard Cosmos-782 also demonstrated that a statistically significant decrease of the mineral content in humeral epiphyses was less expressed than in the tibia (18). This study did not show significant changes in the porosity density or strength of the humerus (7) whereas tibial porosity increased markedly (2).

It is suggested (24) that the lower inhibition of osteogenesis in the humerus as well as the weaker level of other

related changes in the bone may be associated with the initially low degree of its neoformation processes as compared to those in the tibia. This can be accounted for in a different manner as well. The differences in the responses of the humerus and tibial (and femur) bones to the flight exposures can be brought about by the different changes in the contractile properties of the related muscle groups, as illustrated by the soleus and brachialis muscles discussed above.

In other words, it can be assumed that in space flight skeletal bones of the fore- and hindlimbs similarly devoid of the gravitational load are exposed to dissimilar muscle effects differing from those on the Earth. As follows from the study of contractile properties of skeletal muscles, in space flight the effect of the brachialis muscle on the humerus bone can be either increased or changed in its pattern (becoming tonic), thus making up to a certain extent for the deficiency of the support load. At the same time the muscle effect on the hindlimb bones, on the tibial bone in particular, may be markedly decreased due to the above changes in the physiological properties of the soleus muscle (reduction of strength and work capacity, enhancement of the contraction rate), thus aggravating the effect of zero-g.

This notion is supported by the data on the well-known parallelism in the recovery of tubular bones and contractile properties of hind-limb muscles. It was found that during postflight readaptation (Cosmos-1129) the rate of periosteal osteogenesis in the tibia was by 46% ($p < 0.025$) higher than in the synchronous controls, and in the humerus returned to normal (24). A similar rebound-effect was also observed in the normalization of the calcium content in the cortical plate of the femoral diaphysis: immediately postflight the calcium content was 5-6% ($p < 0.001$) lower and 6 days postflight was 4-7% ($p < 0.02$) higher than in the controls (V.E. Novikov, personal communication).

At R + 6 the supercompensation effect in the recovery of contractile properties of glycerinated fibers of hindlimb muscles was seen (S.A. Skuratova, personal communication). The amplitude of the maximum isometric contraction of the soleus preparations was increased (+36%, $p < 0.05$) as compared to the vivarium controls whereas at R + 0 this parameter was significantly decreased (-41.6%, $p < 0.001$). Similar trends were found in the recovery of the extensor digitorum longus muscle. At present it is rather difficult to explain the strength increment of the soleus preparations in this case. It can be associated with other events than the true recovery of the contractile capacity of muscle fibers. However, serious attention should be given to the reconciliation of these data with the dynamics of recovery of tubular bones.

The above data and their interpretation can hardly be considered as sufficient for final conclusions; however, we think they can well be used to advance a hypothesis that the bone response to weightlessness is dictated not only by the total or partial elimination of the gravitational effect on skeletal bones but also by the related changes in muscle forces affecting them.

The discussion of the same factors in the light of the above hypothesis may, in all likelihood, help define the approaches to the solution of the above problem, which is very important both theoretically and practically, viz.: what is the comparative role of the weight-bearing loading and muscle force effects in the normal functioning of bones and, consequently, what is the role of their changes in the mechanism of adaptation of the musculo-skeletal system of mammals to the space flight environment.

REFERENCES

1. Albricht, F., L.H. Burnett, O. Cope, W. Parson. 1941. Acute atrophy of bone (osteoporosis) simulating hyperparathyroidism. J. Clin.Endocrin. 16:28-32
2. Asling, C.W. 1979. Histological examinations of the tibia. In: Effects of Dynamic Factors of Spaceflight on the Animal Body. Ed. Genin A.M., Moscow, Nauka, 157-165
3. Cann, C.E., R.R. Adachi, Emily M. Holton. 1980. Bone resorption and calcium absorption in rats during spaceflight. Physiologist, 23, 6 (Suppl.), 83-86
4. Gazenko, O.G., A.M. Genin, E.A. Ilyin, V.S. Oganov, L.V. Serova. 1980. Adaptation to weightlessness and its physiological mechanisms (Results of animal experiments aboard biosatellites). Ibid: 11-15
5. Gurfinkel, V.S., Ya.M. Kotz, M.L. Shick, 1965. The regulation of man's posture. Moscow, Nauka, 256
6. Hinsenkamp, M., F. Burny, R. Bourgois, M. Donkerwolke. 1981. In vivo bone strain measurements: Clinical results, animal experiments, and proposal for a study of bone demineralization in weightlessness. Aviat.Spac Environ. Med. 52, 2, 95-103
7. Holton, E.M., D.G. Baylink. 1979. Quantitative assessment of selected bone parameters. In: Effects of Dynamic Factors of Spaceflight on the Animal Body. Ed. Genin A.M., Moscow Nauka, 74-79.

8. Itina, N.A. 1974. The neuromuscular system in chordates. In: The Development of the Contractile Function in Locomotor Muscles. Leningrad, Nauka, 239-290

9. Kazarian, L.E., G. Collins, L. Munic, F. Becton. 1980. Strength characteristics of rat spinal columns. Cosmos-1129. Proc. 28th Intern.Congr.Physiol. Sci., Budapest, 1980. 19, Gravitational Physiology. Ed. J. Hideg, O. Gazenko, Budapest, Pergamon Press-Academiai Kiado, 129-138

10. Kitajev-Smyck, L.A. 1974. Reactions of humans and animals to short-term weightlessness. In: Weightlessness (Biomedical Research). Moscow, Nauka, 41-65

11. Korzhujev, A.P. 1976. The Evolution of vertebrates' skeleton and gravity. In: Problems of Space Biology. Moscow, Nauka, 33, 31-46

12. Ljubashevski, N.M., 1980. The Radioisotope Metabolism in Vertebrates' Skeleton. Moscow, Nauka, 255.

13. Minaire, P., P. Meunier, C. Edouard, J. Bernard, P. Courpron, J. Bourret. 1974. Quantitative histological data on disuse osteoporosis. Calcif. Tiss. Res. 17, 57-73

14. Morey, E.R., 1979. Spaceflight and bone turnover: correlation with a new rat model of weightlessness. Bio-Science, 29, 3, 168-172

15. Oganov, V.S. 1980. Investigation of the weightlessness effect on the contractile properties of different skeletal muscles. In: The Nervous Control of the Structural-Functional Organization of Skeletal Muscles. Leningrad. Nauka, 142-162

16. Oganov,V.S., A.N. Potapov. 1979. Study of functions of skeletal muscles in biosatellite experiments. In: Effects of Dynamic Factors of Spaceflight on the Animal Body. Ed. A.M. Genin. Moscow, Nauka, 118-123

17. Oganov, V.S., S.A. Skuratova, A.N. Potapov, M.A. Shirvinskaya. 1980. Physiological mechanisms of adaptation of rat skeletal muscles to weightlessness and similar function requirements. Physiologist, 23, 6 (Suppl.), 16-21

18. Prokhonchukov, A.A., A.G. Kolesnik, N.A. Komissarova, L.L. Novikov, N.N. Sirotkina. 1979. The mineral balance in calcified tissues. In: Effects of Dynamic Factors of Spaceflight on the Animal Body. Ed. A.M. Genin. Moscow, Nauka, 146-148

19. Skuratova, S.A., V.S. Oganov, M.A. Shirvinskaya. 1979. Study of the contractile properties of myofibrillar proteins using glycerinated fibers in the biosatellite Cosmos-936 experiment. In: Proc. 6th USSR Conf. Space Biol. Aerospace Med. Moscow-Kaluga, 67-68.

20. Smith, A.G. 1975. Foundations of gravitational biology. In: Foundations of Space Biology and Medicine. Moscow, Nauka, 2, 1, 141-176

21. Stupakov, G.P., V.V. Korolev. 1979. Changes of bone mechanical properties. In: Effects of Dynamic Factors of Spaceflight on the Animal Body. Ed. A.M. Genin. Moscow, Nauka, 174-179

22. Stupakov. G.P., A.I. Volozhin, V.V. Zasypkin, S.M. Remizov. 1980. Changes of biomechanical properties of rat bones in the 19-day Cosmos-936. Spaceflight. Mechanics of Composite Materials 3, 530-537

23. Szilagyi, T., A. Szoor, O. Takacs, M. Rapcsak, V.S. Oganov, S.A. Skuratova, S.S. Oganesyan, L.M. Murashko, M.A. Eloyan. 1980. Study of contractile properties and composition of myofibrillar proteins of skeletal muscles in the Cosmos-1129 experiment. Physiologist 23, 6(Suppl.), 67-70

24. Wronski, T.J., E. Morey-Holton, W.S.S. Jee. 1980. Cosmos-1129: spaceflight and bone changes, Ibid., 79-82

25. Yagodovsky, V.S., G.P. Gorokhova. 1979. Changes in skeletal bones. In: Effects of Dynamic Factors of Spaceflight on the Animal Body. Ed. A.M. Genin. Moscow, Nauka, 165-174

26. Yagodovsky, V.S., L.A. Triftanidi, G.P. Gorokhova. 1977. Space flight effects on skeletal bones of rats. Kosm.Biol.Aviakosm. Med. 11, 1. 14-20

27. Zenkevich, L.A. 1944. Essay on evolution of the motor system of animals. J. Obshch.Biol. 5, 3, 129-145

28. Zhukov. E.K. 1974. The development of the contractile function of skeletal muscles in ontogenesis. In: The Development of Contractile Function in Locomotor Muscles. Leningrad, Nauka, 89-112

MECHANISMS OF THE EFFECTS OF WEIGHTLESSNESS ON THE MOTOR SYSTEM OF MAN

I.B. Kozlovskaya, Yu.V. Kreidich, A.S. Rakhmanov

Institute of Biomedical Problems, USSR Ministry of Health, Moscow, USSR

Studies of the effects of weightlessness on the motor function and regulation constitute an important line of research in space physiology. Previous investigations revealed a wide spectrum of motor effects of weightlessness including changes in muscle properties (1,2,3), shifts in the sensory systems and reflex mechanisms (5,6), and perceptual and coordination disorders (7, 8, 9). The present-day concepts attribute these changes primarily to the weight-bearing unloading and related muscle atrophy. Clinical and physiological examinations of the motor function carried out in the 140- and 175- day space flights onboard the Salyut-6 station are however at variance with these concepts and point to the multifactorial genesis of the postflight changes: the magnitude and duration of changes in different components of the motor system of the crewmembers were not correlated (10). It is obvious that a better understanding of the mechanisms of weightlessness-induced motor abnormalities requires further accumulation of quantitative data on the state of different components of the musculo-skeletal system after flights of varying duration. This is the purpose of the present investigation.

1. Methods of Research

All crewmembers of the 140-, 175-, 185- and 73-day space flights onboard the Salyut orbital station were investigated according to the same protocol which included methods and tests that allowed quantitative evaluation of the major proprioceptive inputs, i.e., support and muscle, the muscular system, spinal mechanisms and systems of the postural and locomotor regulation.

1.1. Sensory Systems. The state of the support input was determined with respect to the vibrosensitivity threshold of the sole support areas. The highly sensitive to vibration stimulation Vater-Pacini's corpuscles are concentrated in the subcutaneous tissue of the medial and lateral pads of the foot, in the area of the calcanean tuber and the pad of the great toe (11). Using the apparatus Vibrotester, the vibrosensitivity thresholds in the above areas were measured at three frequencies (low - 63 Hz, moderate - 125 Hz and high - 250 Hz).

The state of the muscle input and related mechanisms of spinal regulation was evaluated with the aid of the recruitment curve of the tendon reflex of the gastrocnemius muscle (Achilles T-reflex), showing the reflex amplitude as a function of the stimulation strength. Calibrated taps over the Achilles tendon were applied. The surface electromyographic responses were recorded. The reflex threshold, maximum amplitude and slope of the recruitment curve were analyzed. These parameters of the curve are not interconnected (12) and reflect various properties of the motoneuron pool: excitability of its low threshold (threshold) and high threshold (maximum amplitude) components and population homogeneity (the slope of the curve).

The comparison of the characteristics of the recruitment curve at rest and with the other foot maintaining a dorsal or a planter flexion made it possible to determine the state of cross synergies, i.e., one of the major mechanisms of locomotor activity.

1.2. Muscular System. In order to evaluate the state of calf and femur muscles, the Miotest procedure (13) was used in the examinations of the 140- and 175-day crewmembers. According to the procedure, the major indicator of the muscle function is the electromechanical coefficient describing the ratio of the integrated EMG to the standardized load. As known, the effort made by the muscle correlates linearly with the magnitude of the integrated EMG in the range of efforts amounting to 0-20% of the maximum. With a decline of the contractile properties of the muscle its EMG amplitude, when performing a standardized effort, increases due to increases of the number of motor units recruited, frequency and synchronization of their discharges (14).

Later the protocol of muscle studies was supplemented with isokinetic dynamometry, using the Cybex dynamometer. The tests were performed to measure the speed

and strength of leg muscles, viz., gastrocnemius and anterior tibial muscles, femur and back muscles, with respect to the force moments and the EMG when moving with high (180°/sec) and low (60°/sec) speeds at isometric contractions. In this test (in contrast to all other tests) the averaged prelaunch data characterizing the velocity-strength muscle properties of 15 members of the permanent and visiting crews were used as controls.

1.3. Motor Control Systems state was determined on the basis of stabilographic data and investigations of postural synergies. The stabilographic recordings were conducted according to the standard procedure (15) for 3 min: minute 1 - comfortable posture with eyes open, minute 2 - the same posture with eyes closed, minute 3 - Romberg's posture. In this situation, high frequency oscillations of the body mass centre calculated as a percentage of the total number of oscillations were taken into study.

The stability was also measured in relation to the time of balance recovery in the tests with perturbations of the body balance, generated by pushed of a known force against the subject's chest. It is known that the imbalance which may result from any perturbation is usually prevented and restored by the correction activity of the muscular apparatus. The complex motor reactions precisely arranged in time and space, responsible for the body stability, are termed postural synergies (16). When measuring the amplitude and time of bursts of leg muscles during standing and perturbation tests, it was possible to describe qualitatively and quantitatively the system controlling postural synergies.

These examinations were carried out before and on days 2(3), 5(6), 9(11), 25 and 35(45) after flight. The preflight data were used as controls.

2. Results and Discussion

2.1. Sensory Systems. On return to the crewmembers showed a marked trend for proprioceptive hyperreactivity. This included a pronounced decrease of vibrosensitivity thresholds of the support areas of the foot seen in four cosmonauts with every stimulation frequency (Fig. 1) and a drastic decrease of the thresholds of tendon reflexes. As follows from Fig. 2, the thresholds of the gastrocnemius muscle T-reflex, which preflight averaged 1.2 kg, decreased more than twice postflight. In contrast to the clinical hyperactivity, the threshold decline in the cosmonauts was followed by significant decrease of the maximum amplitude of the reflex responses and the slope of the recruitment curve. Postflight, mechanisms of cross synergies were significantly disturbed: voluntary dorsal and plantar flexions of the gastrocnemius muscle of the other leg, which preflight induced reflex inhibition and facilitation, respectively, did not influence the reflex parameters postflight (Fig. 2).

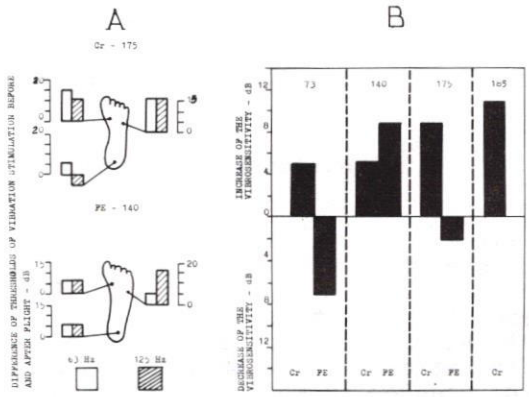


Fig. 1. Changes in the vibrosensitivity of the support areas of the sole of the Salyut-6 crewmembers.
A - data for the 175-day and 140-day crewmembers at frequencies of 63 and 125 Hz, B - data for 7 crewmembers
Ordinate: the difference of thresholds of vibration stimulation before and after flight, dB. Above the line - increase, and below the line - decrease in the vibrosensitivity

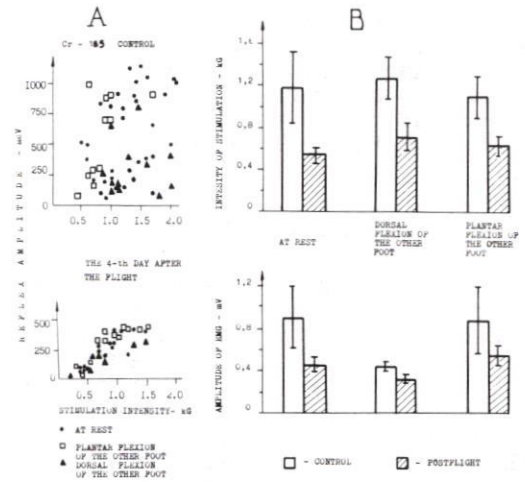


Fig. 2. Characteristics of the T-reflex before and after Salyut-6 flights
A - recruitment curves of the T-reflex of the 185-day Commander before and after flight. - at rest, - with the dorsal and - with the plantar flexion of the other foot, B - averaged parameters of the threshold and maximum

amplitude of the tendon reflex of the Salyut-6 crewmembers before and after flight.

The duration of these changes varied greatly, ranging from 9-11 days (T-reflex thresholds) to 36 days or more (the amplitude of reflex responses and vibrostimulation thresholds).

2.2. Muscular System. The anthropometric and neurological examinations of the crewmembers of the long-duration flights demonstrated no significant changes in the peripheral muscles, except for noticeable atony of the calf muscle rear group and atrophy of long muscles of the back of the 140-day Flight-Engineer and slight subatrophy of the latissimus dorsi muscles of both 175-day crewmembers. The atony was of short duration, its symptoms declined by day 5-6 and disappeared entirely by day 11 postflight.

However, physiological tests showed certain changes in the velocity-strength muscle properties. As follows from Fig.3A, postflight the cosmonauts exhibited a slight strength decrease of the gastrocnemius muscle at the velocities of 0 and 60°/sec and a noticeable strength decline of the anterior tibial muscle at every velocity except for 180°/sec. The reduction of the muscle strength manifested

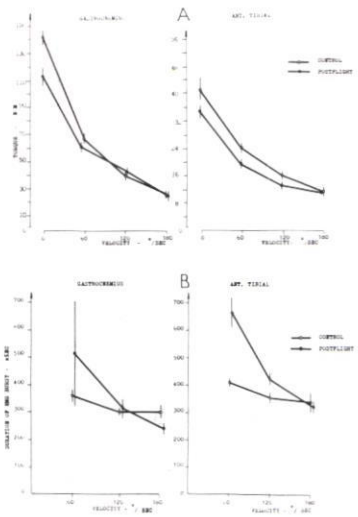


Fig. 3. The velocity-strength properties of the gastrocnemius and anterior tibial muscles in the Salyut-6 crewmembers before and after flight.
Abscissae: the velocity of movements, °/sec. Ordinate: - maximum force moments, N.M., - EMG duration, msec.

not only as a decrease of the maximum efforts but also as a significant increase of the time required for their achievement (Fig. 3B). The use of isokinetic dynamometry of neck muscles in the 185-day crewmembers demonstrated a significant decrease of their strength capabilities observed at every velocity (Fig. 4).

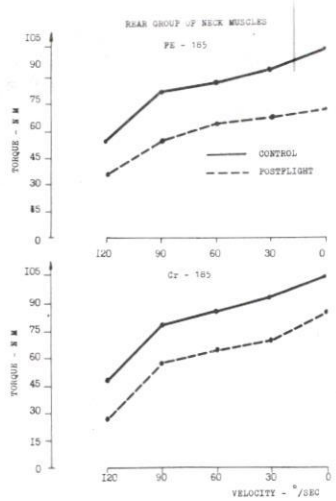


Fig. 4. The velocity-strength characteristics of the rear group of neck muscles in the 185-day crewmembers before and after flight
Abscissae and ordinate as in Fig. 3,A.

These changes were relatively stable and were recorded till the end of the surveillance period that continued for 42 and 45 days in the 140- and 73-day crewmembers, respectively.

2.3. Motor Control Systems. The coordination tests showed significant disorders in the mechanisms of postural regulation in all the cosmonauts. This was manifested as a change in the stabilographic curve, characterized postflight by the predominance of high frequency oscillations of the tremor type, a significant increase of the amplitude of the standing EMG of calf muscles, and an increase of the time of the posture imbalance (up to 23 sec or more) when the subject closed his eyes and took Romberg's posture. The most pronounced changes in the postural stability were seen during perturbation tests. The time of the balance recovery after external disturbances increased significantly, the thresholds of the correction electromyographic responses decreased substantially, and their amplitude and duration increased (Fig. 5).

The duration of coordination disorders in the crewmembers of different flights varied appreciably: it was the longest in the 140-day crewmembers in whom locomotor and postural recovery took more than 42 days, and the shortest in the 175-day Flight-Engineer and the 185-day Commander.

Thus the investigations have demonstrated consistent changes in different components of the motor system and have supported the previously advanced hypothe-

Table 1

Rank parameters of postflight changes in different components of the motor system of the Salyut-6 permanent crewmembers

	73-day		140-day		175-day		185-day	
	Cr	FE	Cr	FE	Cr	FE	Cr	FE
SENSORY SYSTEMS								
Muscle input								
Threshold of the tendon reflex	-2	-2	-3	+2	-3	-4	-4	-2
Amplitude of the tendom reflex	+13	0	-3	-3	-2	0	-3	-1
Support input								
Vibrosensitivity of the sole	+3	-6	+13	+15	+12	-1	+3	
MUSCULAR SYSTEM								
Muscle strength (isokinesis at 180°/sec)								
- flexors	0	0			0	0	0	0
- extensors	0	0			-1	-2	0	+2
Muscle strength (isokinesis at 60°/sec)								
- flexors	-1	0			-3	-2	0	0
- extensors	-1	0			-2	0	0	0
EMG duration								
- flexors	+2	+4				+4	+1	+2
- extensors	+2	+5				+2	+2	0
Electromechanical coefficient								
- extensors			-8	-1	-8	-4		
MOTOR CONTROL SYSTEMS								
Ratio of the evoked EMG amplitude to the perturbation force	+6	+4	+4	-2	+2	+8	+16	+12
Threshold of the evoked EMG	-3	-3	-3	-3	-2	-3	-3	-4
Time of recovery of postural balance	+3	+8	+3	+10	+3	+8	+3	+6

Note: 25% changes as related to the preflight level were taken for rank 1

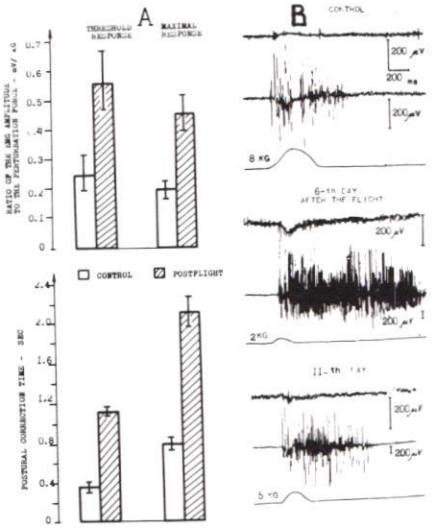


Fig. 5. Characteristics of EMG responses to perturbations in the Salyut-6 crewmembers
A - electromyographic correction responses in the 175-day Flight-Engineer before and after flight. B - averaged ratio of the EMG amplitude to the perturbation force and averaged duration of EMG bursts at minimum and maximum stimulations before and after flight.

sis of their multifactorial genesis. As follows from Table 1, in the 140-, 175-, 185-, and 73-day cosmonauts the level of changes in different components was not correlated; it was not correlated with the flight time either. The level of the coordination changes in the Commander of the 140- and 73-day flights was less expressed after his longer mission. The magnitude of muscle changes in the 185-day cosmonauts was lower than in the cosmonauts of shorter-term flights. The changes in the sensory systems during flights of different duration were comparable.

The comparative analysis of the countermeasures used by the crewmembers of different flights on a preferential basis and the results of motor investigations suggest that the lack of correlation between the level of changes and the flight duration was due to the use of the countermeasures: the magnitude of muscle changes declined distinctly with a greater use of strength exercises; postural and walking changes decreased with a wider application of exercises stimulating postural-tonic mechanisms; hyperreactivity of the support input diminished with an increase of the support loads.

REFERENCES

1. Kakurin L.I., Cherepakin M.A., Pervushin V.J., Effects of Space Flight Factors on the Muscular Tone of Man. Kosm.Biol.Med. 2, 63-68 (1971)

2. Gurovsky N.N., Yeryomin A.V., Gazenko O.G., Yegorov A.D., Bryanov I.I., Genin A.M., Medical Investigations during Soyuz-12, 13, 14 Spacecrafts and Salyut 3 Orbital Station Flights Kosm.Biol. Med. 2, 48-53 (1975)

3. Kozlovskaya I.B., O.P. Koserenko, Yu. V. Kreidich and A.S. Rakhmanov. Effects of 140- day spaceflight on motor system. Proc. VI Conference on space biology, Kaluga, p.18 1979

4. Cherepakhim, M.A., and V.I. Pervushin. Medical support and principal results of examination of the "Soyuz-9" spaceship crew. Kosmicheskaya Biologiya i Meditsina, 4:34-41, 1970

5. Rose, C.E. and J.R. Hordinsky. Crew health: Skylab ½R + 21 day interim report pp25-59 NASA ISC 08706, 1973

6. Gurfinkel V.S., Paltsev V.I., Feldman A.G. and Elner A.M., Changes in Some Motor Functions of Man after Long-term Hypodynamia. In: Problemy Kosmicheskoy Biologii. p. 148, Nauka, Moscow (1974)

7. Chkhaidze L.V., Coordination of Voluntary Movements of Man in Space Flight Environment. p. 133, Nauka, Moscow (1968)

8. Chekirka I.F., and A.V. Yeryomin. Dynamics of Cyclic and Acyclic Locomotions in Soyuz-18 crewmembers after 163-day Space Flight. Kosm.Biol.Med. 4, 9-13 (1974)

9. Kozlovskaya I.B., Yu.V. Kreidich, V.S. Oganov and O.P. Koserenko. Pathophysiology of motor functions in prolonged manned space flights. Acta astronautica, in press.

10. Otelin A.A., Mashansky V.F., Mirkin A.S., Vater-Pacini Corpuscule. p. 403, Nauka, Leningrad (1976)

11. Artemyeva E.N., Kudinova M.P., Zalkind M.S., Kandel E.I., Kozlovskaya I.B., Apparatus of man. Physiol. Cheloveka 3, 913-920 (1977)

12. Bouisset S., EMG and Muscular Force in Normal Motor Activities. In: New Developments in EMG and Clinical Neurology (ed. by J.E. Desmedt), 1, pp. 547-583, Karger Verlag, Basel (1973)

13. Gurfinkel V.S., V.S. Oganov et al. Method of quantitative evaluation of functional state of skeletal muscles of man. Bull. of inventions, N665893 21, 1979

14. Gurfinkel V.S., Kots Ya.M., Skik M.L. Regulation of Human Posture. p.256, Nauka, Moscow (1965)

15. Alekseyev M.A., Asknaziy A.A., Naidel A.V., Smetanin B.N., Regulation of Posture Components of Complex Voluntary Motion of Man. In: Sensory Organization of Movements. 5-12, Nauka, Leningrad 1974

ADAPTATION OF THE RAT SKELETON TO WEIGHTLESSNESS AND ITS PHYSIOLOGICAL MECHANISMS. RESULTS OF ANIMAL EXPERIMENTS ABOARD THE COSMOS-1129 BIOSATELLITE.

David J. Simmons

WASHINGTON UNIVERSITY SCHOOL OF MEDICINE
Department of Surgery
St. Louis, Mo. 63110

In 1965, the U.S.S.R. Biosatellite Cosmos-605 carried rats on a 22d space flight. This marked the first of a series of 5 such investigations. U.S.A. investigators were invited to participate in the last 3 studies-- Cosmos 782, 936, and 1129. A significant fraction of the work was focused upon how short-term(18-22d) periods of hypogravidity affect skeletal tissue dynamics, and whether the observed changes might prove to be reversible during some period of postflight recovery at earth's gravity. As this cooperative program developed over the years, it became possible to ask questions of increasing complexity. The techniques applied to this problem have involved histomorphometric analyses of appositional bone and dentin growth(tetracycline labeling), bone resorption, calcium metabolism(^{45}Ca uptake), bone mechanical testing and maturational studies of bone mineral-matrix moieties. The skeletal materials made available to us provided insights about the effects of null gravity upon elements of the skeleton which subserve diverse functions-- the weight and non-weight bearing bones. This work enabled statements about the mechanisms through which null gravity operates upon the skeleton's cellular compartments, and they have pointed to changes which seem to be specific to space flight in rats.

This paper reviews the results of the skeletal investigations from the final Cosmos-1129 flight, but some valuable perspective is gained if we first cite some of the major observations from earlier flights

COSMOS 782 & 936: The appendicular weight bearing bones(femurs & tibias) suffered reduction in the rates of cortical and trabecular bone growth, in their ash content and mechanical strength(torsion testing), but certain of the forelimb elements were spared the severest effects(1). Quite unexplained was the fact that periosteal growth was diminished while endosteal growth and resorption(osteoclast numbers/marrow diameter)remained unchanged. The Cosmos-936 Biosatellite also included some rats maintained in a 1G centrifuge during flight, and their long bones were spared deleterious consequences which could only be attributed to the 0G of null gravity. It was not until the Cosmos-1129 flight that we began to understand why space flight caused these deficits in bone formation.

COSMOS-1129

MATERIALS AND METHODS

SPF male Wistar rats(83d, avg. 290 gbw) were obtained from the Institute of Experimental Endocrinology of the Slovakian Academy of Sciences. They were divided into three groups of 5-7 animals each;Flight(Group 1), Synchronous Controls(Group-2),and Vivarium Controls(Group 3). The Flight Group was loaded into block modules of 5 cages mounted in a modified Soviet Vostok Spacecraft; they were singly housed. Each unit was about 200mm deep and 100mm in diameter, and had its own environmental controls for light, water and food dispensation. A grating permitted total daily urine and fecal collections. These animals were placed in orbit for 18.5d. During flight,the atmosphere in the spacecraft was maintained at approximately sea level conditions. The temperature varied from 22-25°C, and the light-dark cycle was 12h/12h. Water was supplied ad-lib, and the rats were fed 4X/d with 10g of a nutritionally adequate paste diet. They had been conditioned to this diet for 10d before launch.

The Synchronous Control rats were maintained in a land bound mock-up of the Biosatellite under nearly identical conditions, and they were subjected to simulated stresses of launch and recovery(acceleration, vibration, impact shock etc.). The Vivarium Controls were handled somewhat differently. First, they were multiply housed-(2-3 rats/cage) in standard 55X19.5X33cm cages(Group 3). A subgroup of Vivarium Controls also spent 3, 36h periods isolated in metabolism cages(18X18X12.5cm) during the postflight recovery period. Second, while each cage received 40g/rat paste diet, it was provided in a single feeding during the preflight and orbital phases of the Mission. Group 3 also received 45g of the paste diet once a day during the recovery period.

Groups of 5-7 Flight, Synchronous and Vivarium Control rats were sacrificed within 7-11h,6d and 29d after the spacecraft was recovered. In the period between recovery and sacrifice at 6d and 29d, the remaining Flight and Synchronous Control groups were transferred to larger cages(18X18X12.5cm) and housed in a vivarium. The 6d postflight sacrifice groups were fed the paste diet in 4 divided aliquots. The 29d postflight sacrifice groups were fed an increasing allotment of food(45g/d) and were multiply housed like those animals in the Vivarium control group.

In order to follow the changes in the rates of bone formation during the Flight and Postflight Recovery Periods, the animals were injected with 1.0mg/kg declomycin 3d period to launch, and at 5d and 27d following recovery. All animals were sacrificed 24-48h after injection, and their skeletal parts were preserved either by fixation in absolute ethyl alcohol, or in a mixture of paraformaldehyde, glutaraldehyde and difluorodinitrobenzene for histology and chemistry(jaws, ribs, and long bones), or by freezing for biomechanical tests(vertebral column)(2-5).

In order to follow the changes in the rates of resorption of bone, several different kinds of measurements were taken. Histomorphology provided an examination of osteoclast number and ultrastructure(3,6,7). But, further, the rat's diet contained only ^{40}Ca, thus permitting estimates of daily skeletal calcium losses by neutron activation studies of bone-bound ^{48}Ca eliminated in the

urine and feces(8).

In this report, we mainly compare the differences observed between the Flight and Synchronous Control rats. There were some skeletal consequences of environmental isolation and relative immobilization of animals observed when the Vivarium and Synchronous Controls were compared, but these do not directly address the effects of hypogravidity. The graphs do, however, include some data about the Vivarium Control groups.

RESULTS

Bone Formation(Fig. 1): Histomorphological studies of periosteal alveolar bone surfaces(maxillary and mandibular molar regions) involved in the age-wise migration of the teeth(3), and periosteal suraces of the tibias indicated that there had been a 30-40% reduction in the rate of bone formation and/or calcification during spaceflight(2). Bone growth and trabecular bone mass in the "grooming" elements such as the humerus also decreased about 23%, and there was an increase in the length of diaphyseal growth arrest lines in this bone-- but not in the weight-bearing tibia(2). The deficit in bone formation rates per se was associated with a failure of differentiation of osteoprogenitor cells and their conversion to osteoblasts in the maxillary periodontal ligament facing the modeling side of the sockets(9). Figure 2 shows that in the Flight rats, the frequency distribution of nuclear volumes had been shifted toward the smaller undifferentiated cells(80um^3), with a marked decrease in the larger nuclei which characterize the preosteoblasts(130um^3). The apparent decrease in the osteoprogenitor cell populations in the periodontium was reflected in the long bones by replacement of osteogenic marrow with fat(Fig. 3)(2). There were some exceptions to this generalized picture of impaired bone growth during null gravity. The tetracycline data showed that the non-weight bearing ribs(10) and the portions of the mandibular ramus contiguous with muscle did not exhibit a periosteal growth deficit(3,4). However, other data suggested that in the jaws, at least, the tissues formed during the Flight Period were not entirely normal.

When the mandibular bone was ground to 40um particles and separated on a bromoform-toluene gradient into sp.gr. fractions, 70% of the Ca, P, and hydroxyproline-(HO-Pr) is normally found in the most dense mature fraction(2.2-2.9 sp.gr.). In the Flight rats, there was a 30% reduction in these moieties in the mature fraction, and a corresponding increment in the least dense, most

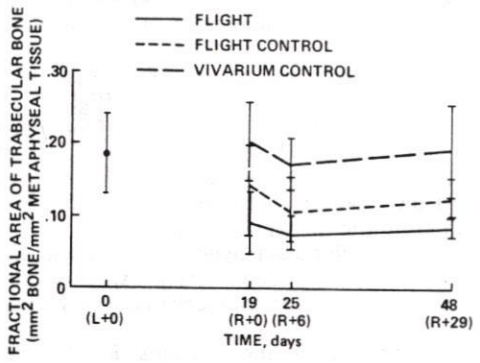


Fig. 1: Trabecular bone volume in the proximal tibial metaphysis vs. time (L+0= at launch, R+0/6/29=Postrecovery days)(from Ref. 10)

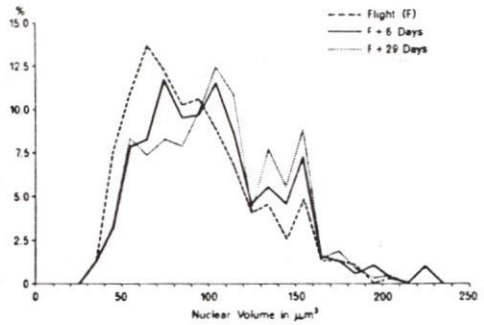


Fig. 2: Frequency distributions of nuclear volume in the maxillary molar periodontium for all Flight rats at Recovery(F) and at different postflight recovery times at IG(Recovery + 6d & +29d). The postflight shifts to the "right" were significantly different(p .001) from the profile recorded immediately after spaceflight.(from Ref. 9).

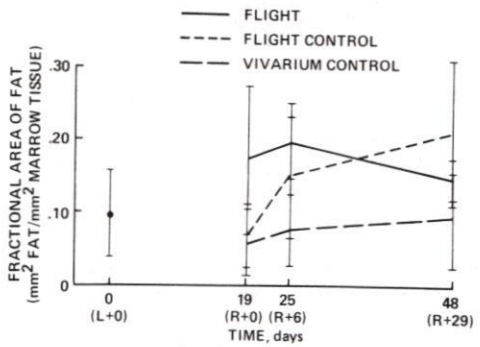


Fig. 3: Fractional area of fat in the bone marrow in the proximal tibial metaphysis vs. time. See legend for Fig. 1 for details of sampling time.(from Ref. 10).

immature fractions(1.3-1.9 & 2.0-2.1 sp.gr.)(Fig. 4). These changes suggested that there might be a distinct deficit in all the bones of animals subjected to null gravity, related to a delay in maturation of collagen and apatite mineral. Ultrastructural studies(TEM) indicted that tibial metaphyseal osteoblasts were less metabolically active than normal; they produced little osteoid and there was a reduced number of mature collagen fibers and mineral nodules(6). Lagging matrix maturation and production of extracellular matrix vesicles-- the probable site of mineral nucleation, were also apparent in epiphyseal cartilage.

Bone Resorption: Whole carcass analyses in a separate group of 5 Flight rats not subjected to histomorphometric analysis indicated that bone mineral(calculated from Ca or P) decreased 22.1% and 13.5% respectively(11). To what extent could this be due to increased osteoclastic resorption? In absolute terms, it could not. The change reflected an impairment of weight gain and tissue growth. The osteoclast census in the tibial metaphysis and mandibular molar region from rats sacrificed immediately after Flight was normal(3,7). Moreover, their ultrastructure revealed signs of diminished cellular activity, i.e., reduced development of ruffled membrane and cytoplasmic vacuoles, and shallow resorption cavities(6).

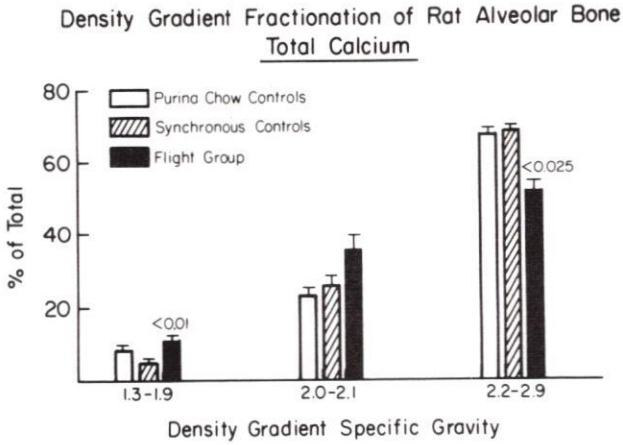


Fig. 4: Density gradient fractionation of rat alveolar bone showing that immediately after Flight, the percent of total bone calcium was increased in the least dense, most immature fraction(1.3-1.9 sp.gr.) and decreased in the most dense, mature bone fraction(2.2-2.9 sp.gr.). The profiles for P and HO-Pr were very similar.(from Ref. 4).

Some resorptive activity did continue during spaceflight, albeit at a reduced rate as evidenced by the somewhat subnormal specific surfaces of resorption in the mandibular molars(3) and levels of ^{48}Ca in the daily fecal collections(8). Bone resorption measured by the excretion of ^{48}Ca was diminished by 20-25%(Flight= 15.7mgCa/d; Controls= 20.2mgCa/d). The reduction was perhaps secondary to a decrease in total body calcium turnover. Thus spaceflight conditions appeared to uncouple the normal balance between bone formation and resorption, with resorptive rates remaining relatively less changed than formation(-30 to -40%). Again, uncoupling could not be discerned by histomorphometric criteria, and the consequences of uncoupling would not be reflected in the mineral:matrix ratios, or seen wherever bone formation rates maintained their normal pace(ribs, jaws with contiguous muscle).

The consequences of uncoupling proved most conspicuous in bones which contained substantial amounts of metabolically active trabecular bone-- the vertebrae. Compressive tests indicated a pervasive loss of bone strength(5), suggesting a decline in the numbers and/or strength of the trabeculae which resist axial loading(Fig. 5). The biomechanical results are consonant with a reduction of trabecular bone mass in the centra, like that in the proximal tibia and humerus(7).

Importantly, most of the histomorphometric, ultrastructural and chemical deficits which developed during exposure to null gravity were partially(vertebral strength) or fully corrected during the 29d postflight recovery period at 1G. It is likely that the process of reversal began as early as 3d postflight. This time corresponds with the predicted minimum of 60-80h required to experimentally shift a (periodontal ligament) nuclear size profile toward the preosteoblast class, thereby restoring the renewing population of osteogenic

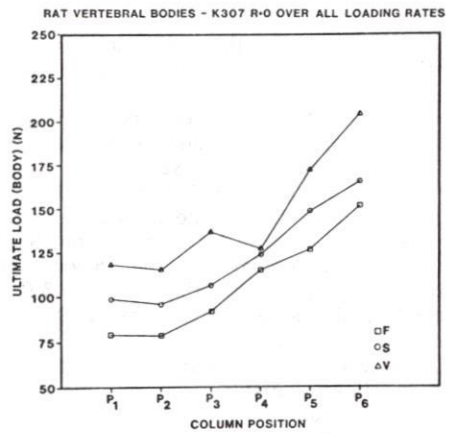


Fig. 5: Plots showing the diminished resistance to axial loading in the vertebral centra of rats sacrificed after Flight. P_1=T2-T4; P_2=T5-T7; P_3=T8-T10; P_4=T11,T12,L1; P_5=L2-4; P_6=L5-7. (from Ref. 5).

cells and their physiologic expression(9). The rate of recovery cannot be expected to be the same for every skeletal element, and our analyses indicate that the affected regions of the jaws and trabecular regions of the weight bearing long bones and vertebrae comprised the least responsive regions of the skeletons.

Teeth: The teeth of the Flight rats seemed to be highly conserved, and hypogravidity did not alter the rates of appositional growth and maturation of the mineral and matrix moieties in the mandibular incisor(4). Fourier analysis of electron microprobe determinations of Ca, P, and S across dentin and enamel traverses also indicated that spaceflight did not affect the biorhythmical components of tooth growth. The daily (circadian) rate of labial dentin and enamel formation(20um and 15um respectively) has 5um ultradian components, and these remained stable in dentin even when the flight protocol required the lighting regimen of a separate group of rats to be shifted by 12h midway through the Mission. In those animals, the biological rhythms of Ca, PO_4 and HO-Pr excretion had normal phase relationships 3d after recovery of the Biosatellite(12). Meal-timing could have acted as an environmental synchronizer(10g aliquots/6h intervals).

COMMENT: As it has developed, the Cosmos Biosatellite Program has provided an incisive description about how null gravity affects the kinetics of the rat skeleton... its cells and mineral metabolism. This information is obviously much more detailed that that which an astronaut population can provide. The applicability of the rat model to the adult human astronaut skeleton has yet to be affirmed in all of its aspects. The rat model is least controversial in terms of the qualitative and quantitative changes in bone matrix formation. It is most controversial in terms of how it relates to the resorption/remodelling rates of bone. The great contribution of the Cosmos Series... and in particular Cosmos-1129, is that it has focused our attention upon critical questions which must be addressed before land-based rodent and primate models can be validated as applicable to the specific environment of spaceflight.

REFERENCES

1. Morey, ER and DJ Baylink. 1978. Inhibition of Bone Formation During Space Flight. Science 201: 1138-1141.
2. Wronski, TJ, E Morey-Holton and WSS Jee. 1980. Cosmos 1129: Spaceflight and Bone Changes. The Physiologist 23: Suppl., S79-S82.
3. Tran Van, P, A Vignery, and R Baron. 1981. The Effect of Space Flight on Osteogenesis and Dentinogenesis in the Mandible of Rats. In: NASA- Final Reports of US Experiments Flown on the Soviet Satellite COSMOS 1129. Suppl. Rept. Experiment K310.
4. Simmons,DJ, JE Russell, F Winter, R. Baron, A Vignery, P Tran Van, GD Rosenberg and W Walker. 1980. Bone Growth in the Rat Mandible During Space Flight. The Physiologist 23: Suppl. S87-S90.
5. Kazarian, LE. 1981. Strength Characteristics of Rat Spinal Columns. Cosmos 1129. In: Adv. Physiological Sciences, Vol. 19, Gravitational Physiology(eds. J. Hideg and O. Gazenko),Pergamon Press, pp. 129-138.
6. Matthews, JL. 1981. Quantitative Analysis of Selected Bone Parameters. Mineralization in the Long Bones. In: NASA-Final Reports of US Experiments Flown in the Soviet Satellite Cosmos 1129., Experiment K-305, Suppl. Rept. 3B.
7. Jee, WSS, TJ Wronski, ER Morey and DB Kimmel. 1981. The Effects of Space Flight on Trabecular Bone in Rats. Am. J. Physiol.(in press).
8. Cann, CE, RR Adachi, and E Morey-Holton. 1980. Bone Resorption and Calcium Absorption in Rats During Spaceflight. The Physiologist 23: Suppl. S83-S86.
9. Roberts, WE and PG Mozsary. 1981. Effects of Weighlessness on Osteoblast Differentiation in Rat Molar Periodontium. Proc. Aeorospace Med. Assoc. Meeting, San Antonio, Tx., May 3-6, 1981, pp.114-115.
10. Wronski, TJ and ER Morey. 1981. Effect of Space Flight on Periosteal Bone Formation in Rats. Am. J. Physiol.(in press).
11. Ushakov,AS, TA Smirnova, GC Pitts, N Pace, AH Smith and DF Rahlmann. 1980. Body Composition of Rats Flown Aboard Cosmos-1129. The Physiologist 23: Suppl. S41-S44.
12. Kwarecki, K, H Debiec and Z Koter. 1980. Rhythms of Electrolytes and Hydroxyproline Excretion in Urine of Rats After Three Weeks of Weightlessness-/Biosatellite Cosmos-1129. The Physiologist 23: Suppl. S34-S37.

CELLULAR ASPECTS OF GRAVITATIONAL BIOLOGY

M.G. Tairbekov and G.P. Parfyonov

Institute of Biomedical Problems, USSR Ministry of Health,
Moscow, USSR

Emergence and development of the organic world on the Earth are inseparably linked with the evolution of the planet itself. Gravity has played an important role in the appearance of the original prerequisites and ensured the conditions required for life. Due to gravity, the solid substrate has formed, the solid, liquid and gaseous phases have separated with respect to their densities, primitive substances have aggregated into more complex compounds, and the polar axis of symmetry has developed. Gravity is involved in cyclic processes of convective shifts of the matter and maintains "the primary order" in nature.

Thus, gravity dependence of living beings on the Earth is clear. However, despite these incontrovertible arguments, the investigations of fundamental biological processes associated with the functioning of a cell as the basic life unit have provided no experimental data to prove direct effects of gravity on the above processes (1).

The interaction of different physical factors with various bodies, living and inanimate, can be realized via the forces which, in regard to their nature, can in principle be divided into three major categories:

1. Nuclear forces that have no direct bearing on biology.
2. Electromagnetic forces involved in molecular interaction, maintenance and breakdown of chemical bonds.
3. Gravitational forces generating the gravity field.

The Earth gravitational field is a force field generated by the gravitational attraction or the force of Earth gravity described by the first of Newton's laws. The gravitational field means gravity distribution on the surface of the Earth. The absolute magnitude of gravity is assumed to be equal to 9.8127 uGall ($1\ ug = 10^{-3}\ erg/cm^2$). Gravity-induced acceleration of the body is constant, being 9.81 m/sec^2. In contrast to other forces, the gravitational force is constant and therefore cannot be a source of energy or exert a direct influence on chemical bonds.

However, as mentioned above, gravity generates certain constraints important for life processes, e.g., phase localization in the environment and the polar axis of symmetry.

The first level of organization of the living matter capable to perceive physical signals from the surrounding medium and transform them into physiological impulses is the cell. The cell is the embodiment of unique properties of life, viz., the abilities to multiply, modify and react to stimulation. Due to specific molecular cell organization leading to a high-order compactness, the cell is capable of efficient replication, transcription and transformation of the information it carries (2).

The functioning of the cell as a relatively independent unit of living matter is determined by the interaction of its major subunits, primarily the nucleus and the cytoplasm. Intracellular interactions are regulated by biomembranes that constitute an inseparable component of every cell organelle and cytoplasm. The cell interaction with its environment is also maintained through cell membranes.

The influx of molecules from the environment into the cell and their outflux back from the cell as well as their transport from one cellular area into another are ensured by two processes: simple diffusion and active transport. In both cases, molecules must pass through membranes either along water-filled channels with a diameter of about 3 Å or via interactions with membrane phospholipids.

In the first case, if molecules with the size less than that of the glycerol molecule are involved, this process is maintained by simple (or facilitated) diffusion, the direction of transfer coinciding with that of diffusion. Under the laws of thermodynamics this process requires no substantial energy expenditures. In other words, the rate of diffusion is linearly proportional to the concentration gradient, needs no molecules-carriers or any energy, except for thermodynamic motion. Diffusion always increases entropy of the cell and tends to deep the cell in equilibrium with its environment.

The study of various cells has demonstrated that almost every one has

a system of facilitated diffusion used to transfer the substances the cell receives from the surrounding medium (3).

In the second case, the process is an active transport of substances (mostly of large biomolecules) directed against the concentration gradient and requiring substantial energy expenditures. In order to perform this work, the cell needs a mechanism acting as a pump at the boundary of water/lipid phases and facilitating molecule penetration into the lipid phase. This is the manner in which compounds that are metabolic intermediates of the cell itself are transported. In the case of an active transport, molecules move against the concentration gradient, while the energy ensuring this process is mainly used to maintain the system in a nonequilibrated state. The aim of this process is to limit the release of substances from the cell into its environment, thus maintaining their cellular concentration at a certain level.

The cell as any other biological system utilizes energy in order to keep itself in a nonequilibrated state for as long as possible. Thus, we can distinguish in the biological system of a cell two opposite thermodynamic processes that have the same molecular basis and different final goals. The first of them is passive transport, i.e., simple diffusion, which equilibrates concentration gradients, increases entropy and gradually brings the system to an environmentally equilibrated state, whereas the second process is active transport that tends to carry the system as far as possible away from the equilibrated state, thus increasing its organizational level. It is generally recognized that a nonequilibrated state which is a mandatory prerequisite of life isolates a biological system from the environment and assures its relative independence. The more sophisticated the biological system, the further it is from the point of thermodynamic equilibrium.

It is very likely that active transport, this advanced mechanism of transfer of substances, is a property that the cell acquired at a later evolutionary stage to support the transition of primitive biological systems to multicellular organisms. To remain in a nonequilibrated state, the cell needs to be continuously supplied with energy and matter (4).

The present-day organisms use as energy sources either the energy of light quantum absorbed by the specialized photosynthetic apparatus of the cell (the privilege of autotrophs!) or the energy released as a result of degradation of organic compounds, products of metabolism, in the course of oxidative phosphorylation in the specialized cell organelles, i.e., mitochondria. In both situations, this is an ATP molecule that serves, so-to-say, as a universal energetic currency with which the cell pays all energy-requiring processes.

The methods of energy transformation in the cell may be either rapid or slow. The methods of substance transfer may be only slow, depending on the rate of diffusion or convection.

In his famous publication, Pollard discusses the laws governing the distribution of molecules (large and small) in e cell of an averaged size (5). Applying Pirri's equation, the author has convincingly demonstrated that in the cells whose size is no more than 1 mm^3 diffusion plays the major part in the distribution of substances. With increase in the cell size, convective processes become more important and, therefore, gravity may affect mass exchange (6).

As known, the largest prokaryotes are no larger than 1 um and the largest eukaryotes - 50 um. There are but few exceptions. For instance, cells of certain algae may be as long as 5 mm and those of myxomycete fungi even longer. The morphofunctional peculiarities of these latter in relation to gravity will be discussed below.

It appears that in order to guarantee life, evolution needed to delineate spatially its major unit, i.e., the cell. In order to function normally, the cell was to be limited in size due to two reasons. First of all, it is necessary to help diffusion to supply every cellular area with nutrient substances with identical probability, since the maximal distance for efficient diffusion is no more than 1 mm (7).

Another factor limiting cell dimensions is related to the mathematical basis (8). It requires that the ratio of the cell volume to its surface be constant and equal to 1:3 or less. If the ratio increases, diffusion fails to maintain reliable transport in the cell. Therefore, to provide effective diffusion the cell should be of such shape and size as not to allow a single point within it be located more than 1 mm from the surface.

The effect of gravity (or weightlessness) can be real if its polar influence is more efficient than the equiprobable diffusion in every direction. This possibility is ruled out for the cell whose volume is no more than 1 mm^3. Therefore, all unicellular free-living organisms are gravity-independent. This implies that a change in the magnitude or direction of gravity, including its nullification (zero-g or weightlessness) cannot influence these organisms. The results of many studies of microorganisms flown onboard space vehicles are in good agreement with the theoretical speculations (9,10). The major conclusion drawn

from these experiments is that weightlessness induces no important changes in spontaneous or induced mutagenesis, growth, development or survival of unicellular organisms.

While the behaviour of individual free-living unicellular organisms in a gravity field (1 g or 0 g) is more or less clear, the peculiar features of the functioning of the unicellular population, its growth and distribution need further study by researchers working in gravitational biology.

Many unicellular populations generate, in liquid media, specific types of distribution which can long be maintained by the gravity-dependent bioconvection (11, 12). As in weightlessness the convective movement is absent, it can be expected that chemical gradients can be equilized and, consequently, concentration gradients in the cell and the rate of its growth can be modified. These speculations may account for the results of certain experiments in weightlessness that revealed differences in the growth rate of cells flown onboard the spacecraft and cultivated on the Earth (13-15).

Weightlessness generates, as a rule, better conditions for both microorganisms and unicellular algae to be cultivated; this is the reason why there is a difference between the space- and ground-based experiments in favour of those former, provided that all other experimental conditions are identical.

It is obvious that potential effects of gravity on the cell, which is a constituent of a multicellular plant or animal organism or an initial stage of the fertilized and cleaving egg in many species of amphibians, fish and birds, may be much more complicated.

This involves, first of all, plant geotropism; it can be said that orientation of major organs of plants in the gravity field is cell-related. The modern theory of the mechanism of gravity effects on plants is, in fact, a synthesis of two concepts: the theory of statoliths advanced by Nemec and Haberlandt, and the auxin theory put forth by Kholodny and Went. Both theories explained the geotropic bending of plant organs as a result of active shifts of the cell content (starch granules and statoliths in the first case, and the growth hormone, auxin, in the second) in response to a gravitational stimulus. Among many investigators that have enriched these concepts mention should undoubtedly be made of Sievers and Volkmann who have made an important contribution to the study of mechanisms of geostimulation at the cellular level (16, 17).

Second, eggs of most representatives of amphibians, fish and birds belonging to the telolecithal organization type are asymmetric in their mass distribution and have a relatively heavy vegetative and a light animal end.

Some experiments in actual weightlessness and clinostatic gravity compensation have demonstrated that these exposures may produce a wide spectrum of abnormalities in cell cleavage due to which early embryogenesis loses its stability. These abnormalities occur only at the earliest developmental stages between the formation of the first furrow separating the cell into the animal and vegetative ends, and the formation of the gastrula (18).

There is however a different point of view, also based on the experimental findings, holding that the animal-vegetative polarity of the cell develops long before the fertilization period (19). It cannot be excluded that the normal development of fertilized eggs in weightlessness requires only a short-term gravitational stimulus.

The plasmodium of myxomycetes, representatives of lower fungi, is a gigantic multinuclear syncytium that may occupy a large surface area of several dozens centimeters. Preliminary investigations of this organism, including its exposure to weightlessness (20), have suggested that gravity may influence its morphofunctional characteristics. It is very likely that such gigantic cells may be used as a very suitable object for gravitational biology investigations.

Thus, it can be postulated that neither weightlessness nor gravity compensation exerts a direct effect on the unicellular organisms, whose dimensions are determined by their environmental constraints. However, weightlessness may affect the cell if it functions as part of a single multicellular organism. This effect is most probable if the cell performs specialized functions related to the orientation of the organism in the gravity field (e.g., cells of the apical meristem of roots of higher plants or specialized cells of the balance organs in animals).

In all cases concerning nonspecialized somatic cells which function as constituents of multicellular organisms, the changes they may develop result from metabolic shifts at the organism level primarily due to changes in energy processes.

REFERENCES

1. Tairbekov M.G., Parfyonov G.P. 1981. Biological investigations in space. Kosm.Biol.Avia Kosm.Med. 2:51-60

2. Lehninger A.L. 1972. Biochemistry New York, 21-28

3. Yost H. 1977. Cellular Physiology New Jersey, 134-136

4. Volkenstein M.V. 1980. Physics and Biology Moscow Nauka, 31-32

5. Pollard E.C. 1965. Theoretical studies on living systems in the absence of mechanical stress. J. Theor.Biol. 8:113-118

6. Kessler I.O., Bier M.Y. 1977. Gravitational dynamics of biosystems. Progress in Astronautics and Aeronautics 52:125-149

7. Bray H.G., White K.S., 1957. Kinetics and Thermodynamics in Biochemistry London 157-159

8. Farish D. 1978. Biology: the Human Perspective New York 31-32

9. Parfyonov G.P., Lukin A.A. 1973. The results and perspectives of microbiological investigations in space. Space Life Sci 4:139-159

10. Parfyonov G.P. 1975. The biological indication of new tracks in space. Foundations of Space Biology and Medicine US/USSR joint publ. Book II. 2:309-339

11. Platt Y.R. 1961. Bioconvectional patterns in cultures of free-swimming organisms Science 133:1766-1767

12. Plasset M.S., Winet H.D. 1974 Bioconvectional patterns in swimming microorganism cultures as an example of Raylight-Taylor instability. Nature 248:441-443

13. Planel H., Tixador R., Nefedov Y., Gretchko G., Richoilley G. 1979. Preliminary results of "Cytos" experiment flown in "Salyut-6". Life Sci and Space Res. 17:139-141

14. Mattoni R.H.T., Keller E.C. 1971. Induction of lysogenetic bacteria in the space environment. In: The Experiment of Biosatellite II, 309-324.

15. Cordium V.A., Polivoda A.B., Manko S.G, Koushin W.J., Zuranov P.V. 1976. The cellular characteristics of Proteus vulgaris cells grown in extremal conditions. In: Organism and Gravity Vilnjus, 198-201

16. Sievers A., Volkmann D., Hendel W., Briegleb W. 1976 Cell polarity in root statocytes in spite of simulated weightlessness. Naturwissluschaften 63:343-348

17. Sievers A., Volkmann D. 1977. Ultrastructure of gravity perceiving cells in plant roots. Proc.Roy.Soc.B. 189:525-537

18. Dorfmann Y.G., Cherdantsev V.G. 1977. The role of gravity in the early development. In: Biological Environment and Developing Organisms Moscow, Nauka, 140-173

19. Scharf S.R.,Gerhart Y.C. 1980. Determination of the dorsal-ventral axis in eggs of Xenopus laevis. Developmental Biology 1:181-198

20. Tairbekov M.G., Parfyonov G.P. et al. 1981. Biological investigations onboard the biosatellite "Cosmos-1129". Adv.Space Res. 1:89-94

EMBRYONIC DEVELOPMENT DURING CHRONIC ACCELERATION

Arthur H. Smith and Ursula K. Abbott

Departments of Animal Physiology and Avian Sciences
University of California
Davis, California 95616 USA

The influence of gravity upon embryonic development has been investigated since Pfluger noted, in 1883, that the first division of frog eggs was parallel to the field of gravity and that abnormal orientation interfered with the developmental process. Subsequent investigations have indicated that embryogenesis is affected by the strength of the gravitational field, as well as its orientation.

We are studying the influence of moderate acceleration fields (1-5 G, with a normal orientation), applied throughout the incubation period, upon the development of the chick embryo. Brief exposure to more intense fields (25-125 G) induces turbulence within the yolk, dispersing the blastoderm and producing a proportionate embryo death [1,2]. The kinetics of this effect indicate a threshold at 8 G, so at our level of treatment, acceleration effects should result from changes within the embryo.

MATERIALS AND METHODS

Chicken eggs are fertilized shortly after ovulation. Cell division begins shortly thereafter and during the 24 hours prior to oviposition the zygote develops to an early gastrula stage (20,000+ cells). Studies of gravitational effects on this developmental period require centrifugation of laying hens which is feasible with an acceleration-tolerant strain of chickens [7] up to fields of 2.5 G. For this treatment we used two large animal centrifuges, 5 m diameter and each with a capacity of 100 hens [4]. Cocks also were included for natural matings -- supplemented as necessary by artificial insemination. Eggs were collected twice daily from the centrifuge, and periodically set, with control eggs laid by un-centrifuged hatchmates, in a small incubator [Montgomery Ward Model 56LZ-864]. Environmental control in this incubator was subject to variation, and where conditions were less than optimal (control hatch <70%) the results were eliminated. For acceleration studies of later periods of development we have utilized commercially available fertile eggs as well as those available from our special flock.

Incubators were developed on an existing 3.6 m diameter centrifuge [3]. Two of these were arranged with 0° of freedom, the eggs being held parallel to the axis of rotation. Since the yolk orients independently in the gravitational field [6], egg turning, which is essential for the first 2 weeks and is normally carried out through 18 days of incubation, was accomplished by rotating them 120° around their major axis at 2-hour intervals. This procedure was used to eliminate movement of the egg within the inertial field potentially causing undesirable oscillation of field intensity. Two other incubators were arranged with 1° of freedom allowing egg orientation parallel to the net field. In these, egg turning was accomplished by rocking the eggs 120° tangential to the radius of rotation. This involves a variation of 0.05 cm in the radius of rotation, causing a variation of $\pm$0.03% in field intensity. Since the results of incubation at 4 G were quite similar in both kinds of incubators, the more complex 0° of freedom procedure was discontinued.

RESULTS

The influence of a 2 G environment upon the first 24 hours of development (during the formation of the egg) is summarized in Table 1. The results are in terms of % survival for each period, which tends to eliminate the influence of earlier mortality. It is apparent that the early acceleration treatment enhanced mortality significantly in the first 8 days of incubation and during the hatching process (18-21 days development). These also are the periods in which most embryonic mortality ordinarily occurs [5]. This enhancement of mortality during usual critical periods is similar to the effects of other sub-optimal environments (e.g., thermal).

The influence of acceleration, applied during incubation is summarized in Table 2. These studies were conducted with eggs procured commercially, since no suitable eggs were available from our acceleration-tolerant flock. No significant acceleration effect is apparent at 2 G, but a dramatic mortality (>80%) occurs during the first 4 days of incubation at 4 G -- and most of this occurs in the first 48 hours of incubation. This indicates that there is a threshold for acceleration effects which is greater than 2 G and at 4 G the interference with early development is marked.

In the eggs laid at 2 G and incubated at 1 G and also those laid at 1 G and incubated during centrifugation there was an increased mortality in the final stage of development (18 days to hatch). Eggs incubated and hatched at 4 G also had a high mortality in the terminal period. This mortality is mostly associated with problems of reorientation and co-ordination of other processes associated with hatching, such as yolk retraction and chorio-allantois regression.

DISCUSSION

The most obvious effects of gravity upon terrestrial animals result from the load imposed upon the whole system. This places greater

TABLE 1. Embryo survival rate by periods (as % of viable eggs entering period ± standard deviation) for eggs laid at 2 G and 1 G and incubated at 1 G.

	Laid at 2 G	Laid at 1 G	$\Delta\% \left[\frac{2\text{ G} - 1\text{ G}}{1\text{ G}} \times 100\right]$	p
(n sets)	(8)	(8)		
(n fertile eggs)	(376)	(450)		
Period:				
Fertilization to 4 days	88.21 ± 6.44	93.38 ± 2.12	-5.5%	<0.05
5- 8 days	93.54 ± 3.79	97.30 ± 3.11	-3.9%	<0.05
9-12 "	98.66 ± 1.15	99.68 ± 0.92	-1.0%	ns
13-17 "	98.55 ± 1.86	99.59 ± 0.78	-1.0%	ns
18 days to hatch	82.58 ± 5.80	89.83 ± 4.47	-8.1%	<0.02
Fertilization to hatch	65.94 ± 3.55	80.95 ± 3.80	-18.5%	<0.001

TABLE 2. Embryo survival rate by periods (as % of viable eggs entering period ± standard deviation) for eggs laid at 1 G and incubated on centrifuge.

Field	1 G	2 G	4 G(0°)	4 G(1°)	4 G ALL
(n sets)	(4)	(5)	(3/1)	(1)	(4/2)
(n fertile eggs)	(377)	(193)	(89)	(25)	(114)
Period:					
Fertilization to 4 days	90.6 ± 5.4	92.9 ± 2.8	22.5 ± 4.9	36.0	25.9 ± 7.8
5- 8 days	98.8 ± 2.4	100.0 ± 0.0	94.3 ± 9.8	100.0	95.8 ± 8.5
9-12 "	98.4 ± 1.3	96.5 ± 3.5	100.0 ± 0.0	100.0	100.0 ± 0.0
13-17 "	98.7 ± 2.0	99.2 ± 1.9	100.0 ± 0.0	100.0	100.0 ± 0.0
18 days to hatch	89.5 ± 7.8	84.3 ± 9.9	*12.5	22.2	*17.4 ± 6.9
Fertilization to hatch	77.5 ± 3.8	74.1 ± 8.8	*3.1	8.0	*5.6 ± 3.5

*Excluding two sets which were at 1 G, 18 days to hatch.

requirements upon the load bearing systems (muscle, bone) generally leading to an adaptive hypertrophy. It also increases the work required for locomotion in the field, increasing the metabolic activity. However, prenatal life is characteristically in a fluid medium, so the load imposed on the organism is greatly reduced. In a buoyant animal, gravitational fields become effective only by interacting with structures with diverse densities.

Our results indicate that the embryo is affected during very early development, especially over the first four days, and during hatching. During the first four days the brain develops as well as the anlage for all other organs. Also, the heart commences to function and the extra-embryonic membranes, which compartmentalize the egg contents form. The latter require a substantial extension and folding of tissue which may be disrupted by the mechanical load. Observations of embryonic abnormalities which occur during chronic acceleration (to be reported later) indicate an inhibition of development of the axial skeleton, which is rarely seen otherwise, a general retardation of embryonic growth, and circulatory problems.

The final stages of development (after 18 days) include the uptake of fluids, transition to aerial respiration and the reorientation of the embryo into a normal hatching position. At 4 G mortality was very high during this period, with a majority of embryos failing to reorient into the normal hatching position.

ACKNOWLEDGEMENT

This research was supported by a NASA Grant: NSG 7493.

REFERENCES

1. Besch, E. L., A. H. Smith and S. Goren, 1965. Effect of accelerative forces on avian embryogenesis. J. Appl. Physiol. 20:1232-40.
2. Besch, E. L., A. H. Smith and M. W. Walker, 1965. Morphological changes in avian eggs subjected to accelerative force. J. Appl. Physiol. 20:1241-48.
3. Kelly, C. F., A. H. Smith and C. M. Winget, 1960. An animal centrifuge for prolonged operation. J. Appl. Physiol. 15:753-57.
4. Kelly, C. F. and A. H. Smith, 1974. Principles of the Biodynamics. II. Experimental Procedures in Gravitational Biology. Aeromed. Rev. USAF School of Aerospace Med. (Brooks AFB, Texas) pp 1-50.
5. Landauer, W., 1967. The Hatchability of Chicken Eggs as Influenced by Environment and Heredity. Monograph 1 (revised). Storrs Agric. Exp. Sta. (Univ. Connecticut, Storrs).
6. Sluka, S. J., A. H. Smith and E. L. Besch, 1966. Orientation in systems with asymmetric density distribution. Biophys. J. 6:175-88.
7. Smith, A. H. and R. R. Burton, 1980. Gravitational adaptation of animals. The Physiologist 23(Suppl):113-14.

SUPPRESSION OF OSTEOBLAST DIFFERENTIATION DURING WEIGHTLESSNESS

W. Eugene Roberts and Peter G. Mozsary
University of the Pacific Dental School
2155 Webster Street, San Francisco, California 94115

and Emily R. Morey, Ames Research Center (NASA)
Moffett Field, California 94035

INTRODUCTION

Marked depression or arrest of bone formation is associated with weightlessness (1). Although the mechanism of this effect is unknown, it probably involves a failure of osteogenic induction.

Periodontal ligament (PDL), the osteogenic interface between tooth and bone, is a well defined cell kinetic model for assessing proliferation and differentiation of stress/strain induced bone formation (2). Based on nuclear size, PDL fibroblast-like cells are a multicompartmental population. In neutral buffered formalin fixed tissue, relatively large nuclei (> 170 μm^3) are characteristic of preosteoblasts, the immediate proliferating precursors of osteoblasts. Cells with smaller nuclei (< 80 μm^3) are the less-differentiated, self perpetuating source of preosteoblasts. At least in PDL, osteoblast differentiation involves a morphometrically detectable increase in nuclear size (< 80 to > 170 μm^3), which is independent of DNA synthesis (2).

The objective of the present study is to determine if weightlessness alters osteoblast differentiation, as evidenced by a change in relative distribution of large to small nuclei in rat molar PDL of the maxilla.

METHODS

In conjunction with the US/USSR Biological Satellite Program, 83 day old, male Wistar rats were flown aboard a modified Soviet Vostok spacecraft (Cosmos 1129). Three groups of seven animals each were killed as follows: a) immediately after 18.5 days of space flight, b) 6 days after flight, or c) 29 days after flight. Each group was divided into three subgroups: 1) flight, 2) synchronous control and 3) vivarium control. Synchronous control rats were subjected to simulated spaceflight with the exception of weightlessness. All tissues were preserved in triple-fix (3).

Maxillary first molars and surrounding periodontium were demineralized in 10% EDTA (pH 7.4) for two weeks, embedded in modified methyl methacrylate, serially sectioned at 3 µm on a Jung heavy slege-type microtome. The mesial root of the maxillary first molar was cut in the sagittal plane, parallel to the long axis of the root. Serial sections were mounted on gelatinized slides and stained with hematoxylin and eosin/phloxine. The region quantitated was a 300 µm length of midroot PDL on the mesial aspect of the mesial root of the maxillary first molars. Under oil immersion at 1000X, the length (2a) and width (2b) of the nuclei of all fibroblast-like PDL cells were measured with an ocular micrometer. Since nuclei in the area studied are 90-95% oriented in the midsagittal plane and are prolate spheroid in shape (width approximating depth, correlation of $r = 0.9$), nuclear volume of each nucleus was calculated according to $V = 4/3\ \pi ab^2$. Volumes for 100 nuclei from throughout the width of the PDL were determined for each animal. Five animals were quantitated for a total sample of 500 nuclei per subgroup. Each nucleus was categorized according to location within the PDL. Zone I is within 25 µm of bone, Zone II is further than 25 µm but within the bone half of the PDL, Zone III is on the cementum side but further than 25 µm from the root surface, and Zone IV is within 25 µm of the cementum surface. As previously described, about 90% of PDL vascularity in this area is located in Zone II.

RESULTS

The alveolar bone surface, adjacent to the area of the PDL studied, was a bone forming surface. Tetracycline labels revealed markedly depressed bone formation during weightlessness. Immediately post flight, mean nuclear volume of PDL fibroblast-like cells was 85.90 ± 19.41 μm^3 (mean standard deviation; $n = 500$), which is significantly ($p < .001$) less than either synchronous (112.69 ± 41.13) or vivarium (101.96 ± 38.10) controls. At 6 and 29 days post flight, no significant differences were noted. Compared to both control groups, frequency distribution of nuclear volume (intervals of 10 μm^3) for the immediately post flight group revealed a marked decrease in the relative number of large nuclei (> 130 μm^3).

PDL width of animals killed immediately after flight was 106.0 ± 2.9 µm (mean ± standard error, $n = 5$), which is significantly ($p < .001$) less than the synchronous control (148.8 ± 3.3), and also significantly ($p < .01$) less than the vivarium control (147.5 ± 7.8).

DISCUSSION

The overall range of frequency distributions (figures 1 and 2) is about 25% less than for previous studies of formalin fixed tissue (2). Since relative distribution of nuclei is quite similar in both instances, this difference probably reflects increased tissue processing shrinkage with triple fix (3) compared to formalin. Thus, preosteoblasts in this study are expected to have nuclei 130 μm^3, while their relatively undifferentiated precursors have a nuclear size of < 80 μm^3.

Compared to either synchronous or vivarium controls, weightlessness depletes preosteoblasts (large nuclei), while numbers of less differentiated precursor cells (small nuclei) are relatively increased (figure 1). This may be a generalized, systemic effect on bone because the PDL/maxillary complex is not weightbearing.

Considering the depleted preosteoblast compartment, previous cell kinetic data in PDL (2) predicts a minimum of 60-80 hours following space flight to reinitiate bone formation, which is essentially identical to the three day estimate calculated from tetracycline labeling of Cosmos rat tibias (1).

Even though PDL width is decreased 29% following spaceflight there is no change in cell density, indicating a net loss of cells during weightlessness. Thus, the preferential decrease in preosteoblasts involves not only a block in differentiation, but a failure of proliferation and/or enhanced cell death as well.

This morphometric study suggests that depleted numbers of preosteoblasts may be an important factor in the inhibition of bone formation during weightlessness.

REFERENCES

1. Morey, E.R. and Baylink, D.J.: Inhibition of bone formation during space flight. Science 201:1138-1141, 1978.
2. Roberts, W.E., Goodwin, W.C. and Heiner, S.R.: Cellular response to orthodontic force. Dent. Clin. No. Amer., 25:(1)3-17, 1981.
3. Corbett, R., Philpott, D.E., and Black, S.: Prolonged fixation studies for space flight. Ann. Proc. Electron Microscopy Soc. Am. 31: 332-333, 1973.

This research was supported by NASA-Ames Research Interchange NCA2-OR588-002, NIH Grant Nos. DE05136, 2 S07-RR-05301 and the Pacific Dental Research Foundation.

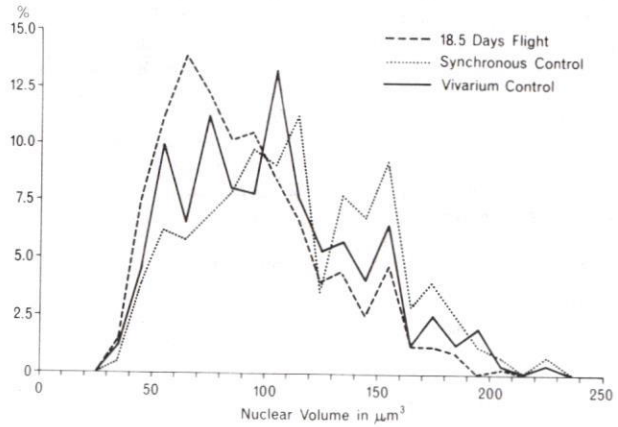


Figure 1. Frequency distributions of nuclear volume for rats killed immediately after 18.5 days of space flight, compared to synchronous (simulated flight) and vivarium controls. All three curves are significantly ($p < .001$) different from each other. Sample size for each distribution is 500 (5 animals X 100 nuclei).

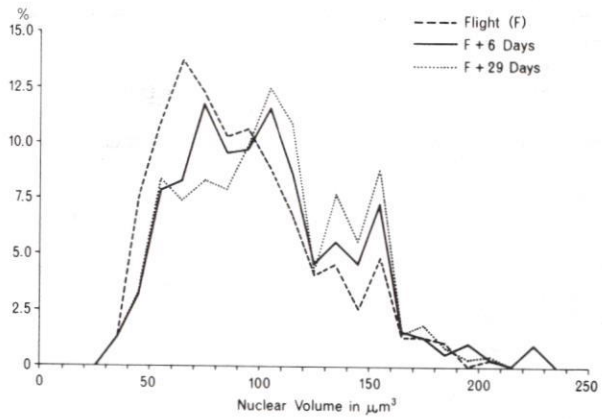


Figure 2. Frequency distributions of nuclear volume for all flight animals. Both the F + 6 days and F + 29 days groups were significantly less ($p < .001$) than the Flight (F) group. Sample size for each distribution is 500 (5 animals X 100 nuclei).

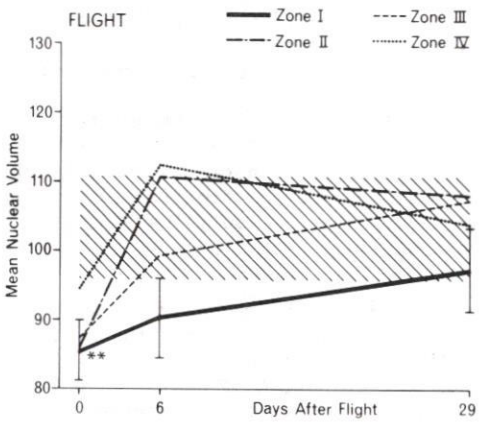


Figure 3. Mean nuclear volume for the four histological zones of the PDL from rats exposed to 18.5 days of weightlessness. The crosshatched area is the vivarium control range, which is the mean ± 1 standard deviation of all four zones combined. Immediately after flight (0 days), zones I, II, and III are significantly ($p < .01$) less than vivarium controls.

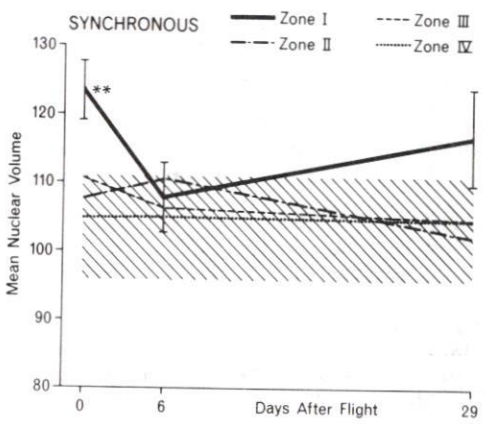


Figure 4. Mean nuclear volume for the four histological zones from synchronous control rats. The crosshatched area is the vivarium control range, which is the mean ± standard deviation, of all four zones combined. Immediately after flight (0 days), mean nuclear volume of zone I is significantly ($p < .01$) greater than vivarium controls.

CLINOSTAT EXPOSURE AND SYMMETRIZATION OF FROG EGGS

George W. Nace and John W. Tremor

Division of Biological Sciences and Center for Human Growth and Development
The University of Michigan, Ann Arbor, Michigan, 48109 and
Biosystems Division, Ames Research Center, NASA
Moffett Field, California, 94035

INTRODUCTION

The unfertilized vertebrate egg is biradially symmetrical. Between fertilization and the first cleavage division it becomes bilaterally symmetrical. This determination of the embryonic axis is the first morphogenetic event and all subsequent morphogenesis is dependent upon it.

One classical hypothesis states that for amphibians the plane of bilaterality is defined by the sperm entry point (SEP) and the animal-vegetal axis of the egg. Following fertilization cortical granules migrate toward the SEP and internal shifts lead to formation of a lip of yolky cytoplasm, the vitelline wall, under the cortex opposite the SEP. These changes lead to the formation of the grey crescent which marks where the posterior-dorsal structures of the embryo will arise. Recent investigations of this matter assign important responsibility for these changes to the action of the sperm aster although this action may be over-ridden by fixing the egg in the gravitational field in such a manner that the gravitational vector is in opposition to the dorsal-ventral axis (2,3,5).

A second hypothesis, also based on amphibians, states that the plane of rotation in the gravitational field becomes the plane of bilateriality (1). Gravitation has also been implicated in axis determination in fish, reptiles, and birds, and may be true in mammals. Tremor and Souza (6) demonstrated by gravity compensation that at suitable clinostat rotation rates and exposure from fertilization until hatching frog embryos develop abnormally.

On one hand, the outcome to be expected of fertilization of vertebrate eggs in microgravity depends upon the relationship between the "SEP" and the "gravity" hypotheses of axes determination. On the other hand, fertilization at microgravity could assist in resolving the differences between these hypotheses.

Since the orientation of unfertilized eggs and the righting of eggs after grey crescent formation do not influence the axes, our attention was directed toward a comparative study of the initial rotation of the fertilized egg; the so-called Rotation of Orientation (R-of-O).

Initial studies were to determine the timing and dynamics of the R-of-O as distinct from inversion rotations, to confirm prior observations (6), and to examine the influence of gravity compensation at periods which might be critical.

METHODS

Northern Rana pipiens and Xenopus laevis maintained in the Amphibian Facility were ovulated and inseminated by standard procedures (4). For descriptions of rotations, eggs in their jelly masses adhering to microscope slides placed in suitable medium at 18°-20°C in Petri dishes were photographed at timed intervals. To determine egg rotation rates these photographs were subjected to the analysis shown in the following figure.

Measuring the rotation rate of an amphibian egg in its jelly mass.

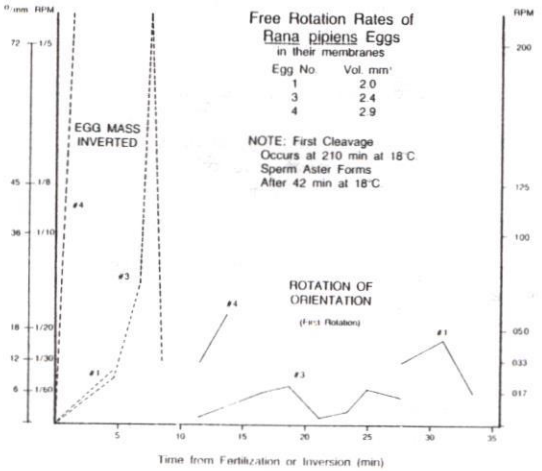
Points D_1, D_2 and B are captured by a digitizer. The computer calculates $S = r - B_1 D$; $\alpha = \text{cosine}^{-1}(s/r)$. Rotation Rate (°/min) = $\Delta\alpha/\Delta t$.

Tests involving gravity compensation were conducted on clinostats provided by NASA. Eggs (10 to 15) were placed within a 5 mm radius of the center of 50 mm Petri dishes. They were flooded with medium, sealed and mounted on the clinostat within 10 min of insemination. Clutch mates in sealed Petri dishes and in open dishes served as controls. Over 2,000 eggs from R. pipiens were tested at 18-20°C at 1/4 or 1 rpm for 1 hr or 4 hr. Development was scored at cleavage, late gastrula, tailbud and hatching stages.

RESULTS

Rotation Dynamics: The following figure shows data on three eggs from among 18 R. pipiens and 8 X. laevis eggs on which R-of-O and inversion rotation data were collected. Before R-of-O the orientation of the eggs was random. None began their R-of-O until 10 min (0.05 normalized to the time of first cleavage for R. pipiens,

0.1 for X. laevis whose cleavage period is 90 min) post fertilization but all completing it by 40 min (0.2 normalized time for R. pipiens, 0.45 for X. laevis). The R-of-O for R. pipiens averaged 1/29 rpm (0.035 ± 0.020) and ranged from 1/200 (0.005) to 1/13 (0.076) rpm. Inversion rotations were much faster, the first averaging 1/17 (0.06), the second 1/8 (0.12) rpm. For X. laevis the corresponding averages were 1/77 (0.013 ± 0.007) rpm for R-of-O and 1/11 (0.09) and 1/9 (0.11) rpm for the inversions. These rates were all less than the effective clinostat rates, 1/4 and 1 rpm.



The variation in rate between eggs of a clutch was greater than the difference between the averages for the eggs of the two species. Egg size was not a factor. The initiation of R-of-O was independent of egg orientation but was completed within 30 min, the rotation period being roughly proportional to egg orientation.

The dynamics of R-of-O was influenced by the shape of the egg in the still unswollen jelly. As they assumed spherical shape the R-of-O proceeded in accordance with considerations of torque and momentum.

Clinostat Observations: Observing R. pipiens and using the clinostat conditions employed by Tremor and Souza (10), i.e. 1/4 rpm from fertilization until hatching (5 days), we found a similar variety and proportion of developmental abnormalities. In shorter exposures to gravity compensation, we found more abnormalities at 1/4 rpm than at 1 rpm, and more at 4 hr exposure (15-20%) than at 1 hr covering the period of R-of-O. Abnormalities included asynchronous cleavage (which was often repaired), exogastrulation, retention of yolk plugs, neural fold and tube defects, projections which suggested localized duplications, partial twinning, stunted growth and death. Some early defects were overcome, thus demonstrating the retention of regulatory ability.

DISCUSSION

Both the SEP and gravity influence early frog egg development (2,3,5,6). The SEP has been directly related to axis determination; gravity by implication as reorientation yields modified axes and abnormalities such as duplications. The period of R-of-O, i.e. before aster formation, seems to be critical. Gravity compensation for 1 hr during the R-of-O yields fewer abnormalities. We hypothesize it changes the axes and that return to normal conditions permits regulation. Longer exposure yields more abnormalities, perhaps by perturbating both the action of the aster and regulation. The normal correspondence of the plane of bilaterality with both the SEP and the R-of-O planes suggests a relationship between the SEP and the R-of-O; either the SEP influences the plane of the R-of-O or tensions in the vitelline membrane caused by torsion of the egg in the gravitational field establishes a preferential site for the SEP.

ACKNOWLEDGEMENTS

Supported in part by grant NAGW-29 from NASA, Washington, and contract NAS2-10945 from NASA, Ames. The assistance of D. Hammond, A. Mendel and K. Metzger, all honors students in various phases of this study is gratefully acknowledged.

REFERENCES

1. Ancel, P. and P. Vitemberger. 1948. Recherches sur le determinisme de la symetrie bilaterale dans l'oeuf de Amphibiens. Bull. Biol. Fr. Belg. (Suppl.) 31:182.

2. Gerhart, J, G. Ubbels, S. Black, K. Hara, and M. Kirschner. 1981. A reinvestigation of the role of the grey crescent in axis formation in Xenopus laevis. Nature 292:511-516.

3. Kirschner, M.W. and J.C. Gerhart. 1981. Spatial and temporal changes in the amphibian egg. BioScience 31:381-388.

4. Nace, G.W., D.D. Culley, M.B. Emmons, E.L. Gibbs, V.H. Hutchison and R.G. McKinnell. 1974. "Amphibians: Guidelines for the Breeding, Care and Management of Laboratory Animals". I.L.A.R. (NAS/NRC). Washington, DC. 150 p.

5. Radice, G.P., A.W. Neff, G.M. Malacinski. 1981. The intracellular responses of frog eggs to novel orientations to gravity. This Symposium.

6. Tremor, J.W. and K.A. Souza. 1972. The influence of clinostat rotation on the fertilized amphibian egg. Space Life Sciences 3:179-191.

THE INTRACELLULAR RESPONSES OF FROG EGGS TO NOVEL ORIENTATIONS TO GRAVITY

G. P. Radice,* A. W. Neff,+ and G. M. Malacinski*

*Dept. of Biology and +Dept. of Medical Sciences,
Indiana University, Bloomington, Indiana U.S.A. 47405

Fertilized frog eggs normally keep a constant orientation to gravity by rotating within a fluid filled space to keep their heavier vegetal hemisphere down. Under these conditions, cytoplasmic movements occur that establish the future dorsal side of the embryo opposite the point of sperm entry. Irradiation with ultraviolet light (UV) can relocate the dorsal side to a novel site and also lead to neural (dorsal) defects. We have found that multiple short doses of UV are as effective as a single large dose in producing neural defects. Furthermore, 180° rotation (inversion) of irradiated eggs reduces the UV effect. Since yolk platelets may be the gravity sensing mechanism, we studied their size, density, and distribution in normal and inverted eggs. Large platelets are denser and are primarily in a distinct zone in the vegetal hemisphere, whereas small platelets are less dense and occur in the animal hemisphere. When inverted, the large platelets flow as a coherent mass into the animal hemisphere, and partially displace the small platelets. Apparently, inversion rearranges cytoplasmic components necessary for later neural development into an appropriate configuration.

Amphibian eggs have unique advantages for studying gravity perception in single cells. Before fertilization, yolk platelets are distributed within the egg with a distinct polarity: small platelets in the pigmented animal hemisphere, and large platelets in the unpigmented vegetal hemisphere. The unfertilized egg is radially symmetric about this animal/vegetal axis, and the axis itself is oriented randomly with respect to gravity.

Within minutes after fertilization, the vitelline membrane lifts from the egg surface and the egg rotates within the perivitelline fluid to maintain the heavier vegetal hemisphere down. Following this rotation, but before the first cell division, the egg loses its radial symmetry as a single, dorsal/ventral (d/v) axis is determined, with the dorsal side opposite the point of sperm entry. The position of the d/v axis can be relocated, however, by tilting the egg on its side. Presumably, novel orientation to gravity rearranges components of the cytoplasm that establish the dorsal/ventral axis.

We have studied this effect with a combination of approaches. We had shown previously that if the vegetal hemisphere of fertilized Xenopus laevis eggs is exposed to a single dose of ultraviolet light (UV, 254 nm), the dorsal side formed at random sites, rather than opposite to sperm entry. Moreover, if these embryos were allowed to develop, many displayed abnormal neural (dorsal) development, including microcephaly (5). Dose response curves indicated that multiple UV targets exist close to the surface of the egg. Whether those targets require a single, heavy dose of UV for inactivation was tested by employing low dose pulses of UV. The effects of multiple doses of 5 x 10^3 ergs/min/cm^2 were compared with single heavy doses of 10 x 10^{-3} and 15 x 10^3 ergs/min/cm^2. By employing a numerical scoring system (1), the "average UV effect" was calculated. Several repetitions of the experiment revealed that multiple doses generated quantitatively the same effects on neural morphogenesis as single, heavy doses (Fig. 1a). These observations are consistent with the multiple target model of the action spectrum data (1).

The effects of UV on neural morphogenesis can be corrected by rotating the irradiated egg 90° off its natural equatorial axis (1). An experiment was performed to determine whether complete inversion (180° rotation) was also capable of correcting the UV syndrome (Fig. 1b). Inversion clearly is capable of preventing the UV syndrome, with the average effect reduced from 2.4 to 0.8, when compared with irradiated but noninverted eggs. Inversion, like the 90° rotation, corrects the damage caused by UV irradiation, presumably by rearranging components, which are later required for neural morphogenesis, into their proper configuration.

Since yolk platelets are known to flow in artificially inverted eggs (2), they are a prime candidate for the gravity sensing mechanism. But the physical properties of the platelets have not been carefully examined. We have developed a metrizamide gradient system for isopycnic centrifugation of yolk platelets to answer the following questions: 1) do yolk platelets display uniform density? and 2) do the "natural" cytoplasmic localizations of yolk platelets reflect their density? Although yolk platelets have been isolated on sucrose gradients and characterized biochemically, these techniques removed their bounding membranes, and stripped off the superficial layer (3,4). In our experiments, metrizamide, because of its high density, low viscosity, and failure to cause platelet aggregation, was used to form continuous gradients in Ca^{++} and Mg^{++} free, phosphate buffered saline (PBS), with 1mM ethyleneglycol-bis-β-amino ethyl ether) N,N'-tetraacetic acid (EGTA).

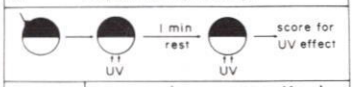
Typically, 4 fertile eggs or 8 halves of eggs were disrupted by repeated pipeting with a narrow bore pipet in 0.5 ml PBS, 2mM EGTA, and then disrupted again after addition of 10% metrizamide in PBS. The preparation was layered on top of the gradient and centrifuged at 30,000 x g for 90 min at 5° C. The bands were collected by piercing the sides of the tubes and analyzed by light microscopy for purity and sizes of platelets. Platelets were judged to be one of three sizes: large (>8 μm), medium (4-8 μm), or small (<4 μm). In a typical experiment, in the region between 35% and 45% metrizamide, there are at least 3 bands of relatively pure, intact yolk platelets with complete superficial layers but without bounding membranes (Fig. 2a). Band 1 contained 35% large, 35% medium, and 30% small platelets; band 2 had 7% large, 23% medium, and 70% small; and band 3 had no large, 10% medium, and 90% small platelets. The proportions vary between batches of eggs, but it is clear that yolk platelets are not all of uniform density, but rather occur in at least three density classes. Small platelets predominate in the low density bands, and large platelets predominate in the high density bands.

To see whether platelet density corresponds to location in the egg, animal and vegetal halves of fertile eggs were centrifuged (Fig. 2b). Band 13 is the major band in the vegetal half. Also, band 1 of the animal half has fewer platelets and only 9% are large, whereas in band 1 of the vegetal half 40% of the yolk platelets are large. Thus, the smaller animal half yolk platelets are mainly distributed to the lower density bands, and the larger vegetal half yolk platelets are mainly distributed to the higher density bands. The simplest interpretation is that the yolk platelets in the intact egg may be localized according to their intrinsic densities.

To see how these platelets rearrange when eggs are given abnormal orientations to gravity, groups of eggs were inverted for 10, 20, 30, or 40 min after fertilization, a treatment that effectively relocates the dorsal/ventral axis (2). They were then fixed in 3% glutaraldehyde, 2% formaldehyde, 2.5% dimethyl sulfoxide in 0.1M phosphate buffer, dehydrated through increasing concentrations of ethanol, and embedded in Embed 812. Semithin (0.5 μm) midsaggital sections were stained with 1% toluidine blue and examined by light microscopy. The minimum time that eggs must be rotated to detect dislocation of platelets was about 10 min. The general pattern of movement that emerged was a mass shift of the vegetal cytoplasm toward the animal half along the cortex of the egg. The large platelets did not appear to mix with the smaller platelets of the animal cytoplasm, but rather moved as a coherent mass or zone. Also, in no case did the vegetal cytoplasm completely displace the animal cytoplasm. Unfertilized eggs, which are insensitive to abnormal orientations to gravity, showed no detectable displacements when inverted for similar lengths of time.

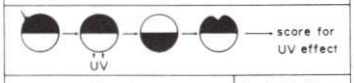
Taken together, these data are consistent with both previous embryological literature and a model in which amphibian eggs sense gravity by the location of the large yolk platelets and associated cytoplasm. Their position during the time between fertilization and first cell division influences the location of the dorsal/ventral axis.

a. Experimental Design



Batch	results (average UV effect)		
	expt. 1	expt. 2	expt. 3
pulse	3.3	3.2	4.0
constant	3.2	3.1	3.8

b. Experimental Design



Batch	Av. UV effect
control (normal orientation)	2.4
experimental (inverted)	0.8

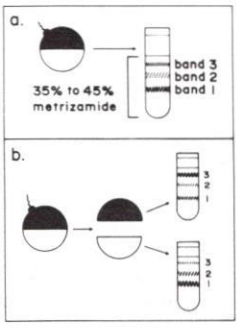


Fig. 1. a. Multiple pulses of ultraviolet light (UV) are as effective as a single large dose of light in producing neural defects. b. Inversion of irradiated eggs corrects the UV effect.

Fig. 2. a. Centrifugation of isolated yolk platelets in metrizamide gradients produces three bands. b. Centrifugation of platelets from the animal or vegetal half of eggs also produces three bands, but platelets from the animal half are mostly less dense than those from the vegetal half.

REFERENCES

1. Chung, H.-M., and G. M. Malacinski. 1980. Establishment of the dorsal/ventral polarity of the amphibian embryo: Use of ultraviolet irradiation and egg rotation as probes. Develop. Biol. 80: 120-133.
2. Gerhart, J., G. Ubbels, S. Black, K. Hara, and M. Kirschner. 1981. A reinvestigation of the role of the grey crescent in axis formation in Xenopus laevis. Nature 292: 511-516.
3. Jared, D. D., J. N. Dumont, and R. A. Wallace. 1973. Distribution of incorporated and synthesized protein among cell fractons of Xenopus oocytes. Develop. Biol. 35: 19-28.
4. Wallace, R. A., and S. Karasaki. 1963. Studies on amphibian yolk: the isolation of yolk platelets from the eggs of Rana pipiens. J. Cell Biol. 18: 153-166.
5. Youn, B. W., and G. M. Malacinski. 1980. Action spectrum for ultraviolet irradiation inactivation of a cytoplasmic component(s) required for neural induction in the amphibian egg. J. Exptl. Zool. 211: 369-377.

This research was supported, in part, by NASA grant No. NAGW-60 to G.M.M., and a NASA Research Associate Award to G.P.R.

GRAVITY SENSING SYSTEM FORMATION IN TADPOLES (RANA TEMPORARIA) DEVELOPED IN WEIGHTLESSNESS SIMULATION

Jürgen Neubert

DFVLR, Institut für Flugmedizin, 5ooo Köln 9o, F. R. G.

ABSTRACT

The gravity sensing system of stage 23 (33 days old) and stage 26,5 (55 days old) tadpoles grown in weightlessness simulation on the fast running clinostat was examined electronmicroscopically. The sensory epithelia (utricular and saccular maculae) of those animals are significantly more vacuolized than maculae of synchronous control tadpoles developed in 1g conditions. Fragments of cell organelles as mitochondria and endoplasmic reticulum were identified in the vacuoles. Further on it could be demonstrated that the growing frog organism after transferred from long duration zero g simulation into normal terrestrian gravity is able to regenerate the significant malformations in the sensory epithelia within some days.

INTRODUCTION

The paper presents actual results of ongoing electronmicroscopic studies about the influence of extended weightlessness simulation on the microstructure of the gravity sensing system in the vestibular apparatus of different developing stages of frog embryos and tadpoles. Earlier experiments had manifested that zero g simulation over more than 7 days results in significant vacuolization of the sensory maculae. The main objectives of this report were to study possible adaptation mechanisms in the experiment animals during long duration zero g simulation which could be documented as diminuation of the characteristic vacuolization. On the other hand, if no adaptation mechanisms could be observed during altered gravity conditions, are the structural changes reversible in tadpoles which were transferred from the simulation conditions into normal terrestrial gravity ?

MATERIALS AND METHODS

Optimal conditions provided, a truly functional zero g state can be simulated for small biological objects using the fast running clinostat [1,6]. The classification of the different developmental stages was defined as in the Normal Tables of Kopsch [2]. The simulation period was started immediatly after artificial fertilization of frog eggs. Rotation speed was between 9o - 1oo rev/min. depending on the developmental stage (size) of the animals. Dechlorinated tap water of 14 $\pm$ 1°C was used. Control animals were allowed to develop normally at 1g in the same aquaria. To answer the question of possible regeneration mechanisms in the frog organism resulting in diminishing of vacuolization, stage 23 tadpoles were allowed to grow in 1g till stage 25 (43 days old) after the 33 days developing period under zero g conditions. Fixation of specimens for electronmicroscopy is described in References [3, 4, 5].

RESULTS

The results of experiments with stage 23 respectively stage 26,5 tadpoles grown in zero g simulation for 33 respectively 55 days revealed that also in these stages essential ultrastructural alterations in the maculae tissues of the gravity sensing systems could be documented. As already reported in earlier papers [3, 4, 5] with regard to junior tadpoles the sensory maculae ultrastructure was characterized by a significant aggregation of vacuoles. The vacuoles were concentrated below sensory cells. In the vacuoles fragments of cell organelles like mitochondria and endoplasmic reticulum could be found. The zones of vacuolization are only present in those sectors where sensory cells and a covering otolith membrane could be observed (Fig. 1 a, b).
The ultrastructural analysis of the sensory maculae of animals which were allowed to develop under normal gravity after an extended stay of 33 days in the simulation device for 1o days (till stage 25) shows that the vacuolization which is significant in stage 23 tadpoles has dramatically diminished and has become unsignificant. No fundamental differences in the microstructure of sensory epithelia tissues between these and normally developed stage 25 tadpoles could be found (Fig. 1 c, d).

DISCUSSION

The most remarkable result of the experiment series described in this report is the ability of the tadpole organism to

regenerate ultrastructural malformations of the gravity sensing systems sensory maculae. These findings coincide with the behavior patterns of animals grown up in simulated weightlessness and then transferred to 1g. They cannot adopt immediately the changed sensations, they swim in typical loops. Animals of this developmental stage need some days for accomodation (normal straight on swimming patterns). I believe it is more than only a conjecture to construct a connection between unusual behavior and vacuolization respectively between a regeneration period resulting in normal ultrastructure of sensory maculae and normal swimming behavior of those tadpoles.

REFERENCES

1. Briegleb, W., Schatz, A. and J. Neubert. 1976. Umschau Wiss. und Techn., H. 19: 621 - 623.
2. Kopsch, F. 1952. Die Entwicklung des braunen Grasfrosches Rana fusca Roesel. Thieme, Stuttgart.
3. Neubert, J. and W. Briegleb. 1977. In Life Sci. Res. in Space, Oser, H., Burke, W. R., Guyenne, T. D. (Eds.). ESA SP 13o, Paris: 165 - 168.
4. Neubert, J. 1979. Aviat. Space Environ. Med., Vol. 5o, No. 1o: 1o58 - 1o61.
5. Neubert, J. and W. Briegleb. 198o. Adv. Space Res., Vol. 1 No. 14 (Life Sci. and Space Res.) COSPAR, Pergamon, London: 151 - 157.
6. Schatz, A., Briegleb, W. and J. Neubert. 1973. DGLRM Prepr.: XXI. Int. Congr. Aviat. and Space Med., München 1973: 113 - 114.

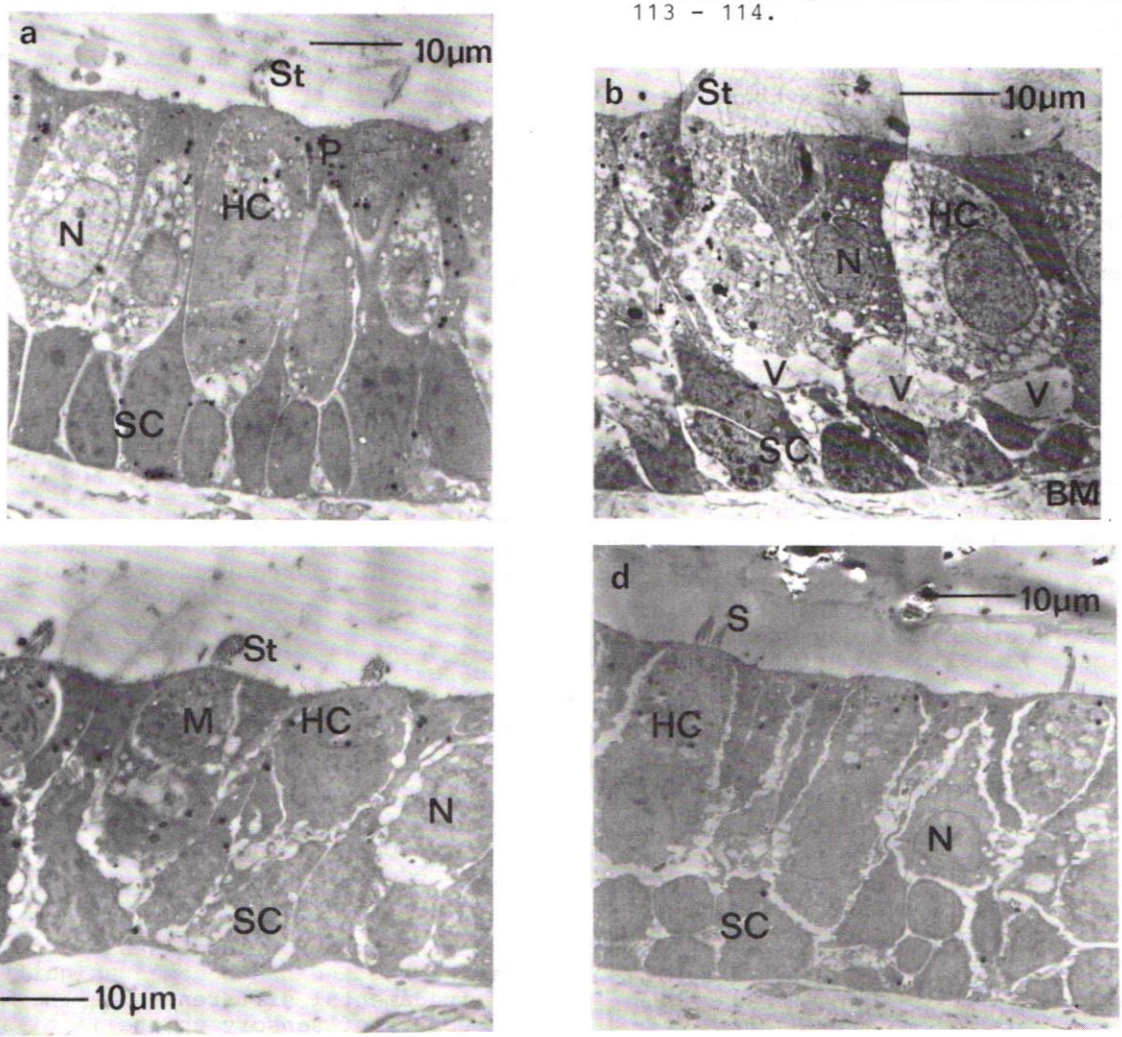


Fig. 1. Macula utriculi of developing tadpoles. a) stage 23 control. b) stage 23 developed from egg fertilization under zero g simulation. c) developed from egg fertilization till stage 23 under zero g simulation and then in 1g till stage 25. d) stage 25 control. BM Basilar membrane, HC Hair cell, M. Mitochondria, N Nucleus, P Pigment, St Sterocilia, V Vacuole, SC Supporting cell.

SHORT TERM GRAVITY EFFECTS ON VOLUME HOMEOSTASIS IN MAN: ASSESSMENT OF TRANSVASCULAR FLUID SHIFTS AFTER GRADED TILT

H. Hinghofer-Szalkay, T. Kenner, and M. Moser

Physiologisches Institut der Universität
Harrachgasse 21
A-8010 Graz, Austria

INTRODUCTION

The mass density of whole blood and of its components (cells, plasma), as well as the density of extravasal fluids (lymph, interstitial fluid), depend on the protein concentration of these fluids. In the biological range, a strongly linear relationship exists between density and protein concentration (PC; both given in g/l), with an increase of 1 g/l PC yielding a density elevation of approximately 0.27 g/l (1).

Typical density values at 37°C are: Plasma density (PD): 1020 g/l (according to a PC of approx. 73 g/l); and blood density (BD): 1050 g/l (according to a hematocrit of approx. 0.42). Human plasma ultrafiltrate has a density amounting 1000 g/l approximately.

Recently, the use of the mechanical oscillator technique (MOT) for continuous blood density recordings (3,4) and density determinations on human blood and plasma microsamples (2) was introduced by our group. The MOT enables rapid measurements on 70-μl-samples with an accuracy and resolution of 10^{-2} g/l.

An increase of the angle between the body axis and the horizontal plane yields a net outward filtration of relatively protein-poor fluid and hemoconcentration, whereas inward movement of fluid and hemodilution follows tilting towards supine position. In other words, blood and plasma density are elevated by standing, and decreased by lying down.

We investigated the time-course and magnitude of these density alterations in blood and plasma of resting humans. Using a two-compartment approach, we additionally calculated the density of the fluid shifted out from or into the circulating blood.

Tilt table experiments are a suitable model for studies dealing with gravitational forces acting on the cardiovascular system. Detailed information about effects of gravity on the body fluid distribution dynamics could be gained by our investigations.

METHODS

The MOT is based on the determination of the resonant frequency of a mechanical bending-type oscillator, made of a U-shaped hollow glass tube, containing the sample the density of which is to be determined. The tube is tightly fixed on both ends and stimulated by electronic means to vibrate perpendicularly to the plane of the U. It is housed in a double walled glass tube. The inner one sorrounding the vibrator is filled with hydrogen gas which provides high heat conductivity and small damping due to low viscosity. The thermostatisizing liquid circulates throughout the outer tube.

The excitation system is formed by a feedback loop containing an infrared light system sensing the motion of the oscillator, and an electromagnetic device applying the stimulation force to the tip of the oscillator.

The resonant frequency f is given by the formula:

$$f = \frac{1}{2\pi}\sqrt{\frac{c}{Mo + \rho V}}$$

where c is the constant of elasticity, Mo the mass of the empty oscillator, ρ the density, and V the volume of the sample.

Rearrangement of this equation yields

$$\rho = A\left(\frac{1}{f^2} - B\right)$$

where A and B are constants characterizing the individual oscillator and including c, Mo and V. A and B can be determined by measurement of f after filling the system with air and water (with known density), respectively, and set by means of a thumb-wheel swith. A microprocessor-based calculator then converts f into ρ.

The samples are prepared in the following way: In the course of the tilt table experiments, blood is taken every 6 minutes from both ear lobes of the test person synchronously. Blood is used for direct density and hematocrit measurements immediately after sampling as well as for consecutive plasma preparation. In the latter case, 70-μl-polyethylene tubes are prepared with 1 μl special heparine solution, having a density of approx. 1020 g/l, at the tip just before sample collection. Then the tubes are filled with blood, sealed, ans coated with glass tubes. After centrifugation, the tubes are cut near the cell-plasma interface, and plasma is withdrawn into plastic connectors mounted on tuberculine syringes. Thereafter, the plasma samples are ready for densitometry and, eventually, additional measurements (like electrophoresis).

Different test protocols were used, including sudden alterations from supine to standing position, and vice versa, graded alterations in 0.1-sinus steps, and combinations of head-up tilt (HUT) and head-down tilt (HDT) maneuvers. From BD and PD alterations in certain test periods, the related density value of the filtrate was calculated.

RESULTS

As a typical example, fig.1 depicts the effect of tilting a test person from -12° (this position was held before onset of sampling for 20 min) to +90°, and back to 45°, in 0.1-sin-steps lasting 12 minutes each, on plasma and blood density.

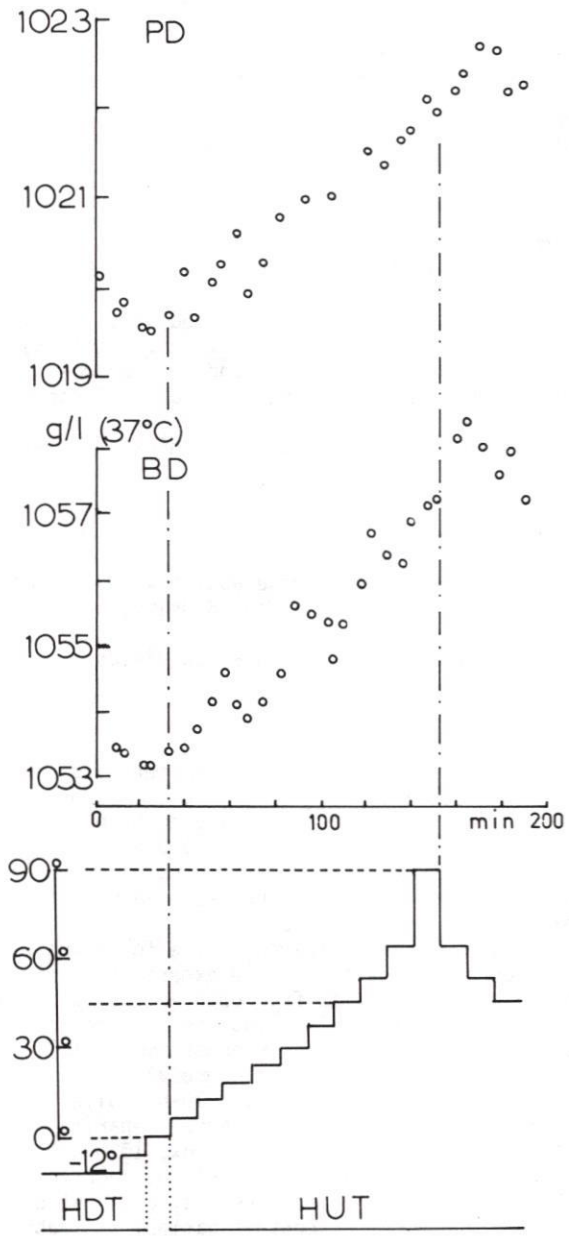


Figure 1
PD = plasma density, BD = blood density, HDT = head-down tilt, HUT = head-up tilt

Similar experiments on 7 male test persons (age: 19-22) yielded the following average differences between steady-state upright and supine values: PD: 3.14 + 0.42 (S.D.) g/l (according to a mean change of PC amounting 11 g/l), and BD: 5.76 ± 1.03 (S.D.) g/l (according to a mean change in hematocrit of 0.07).

The mean filtrate density (FD) from these experiments was calculated to be 1006.5 g/l for outward filtration (increasing tilt angle) and 1005.5 g/l for inward filtration (decreasing tilt angle).

Additionally, in a second group, 8 from 9 test persons showed a slight increase in PD (so far as measured, also in BD) during HDT (-5 to -12°), compared to the respective 0°-steady-state values.

DISCUSSION

From fig.1, it can be seen that 1) a fairly linear relationship exists between the sine of the tilt angle and PD resp. BD, according to the hydrostatic load acting on the capillaries of the dependent parts of the body, 2) a considerable delay occurs in PD- and BD- time course, related to the actual body posture, 3) HDT leads to density increase relative to 0° values similar to the effect of the respective positive angles. This means that during HDT, a net outward shift of fluid takes place as during HDT, probably into headward regions like the shoulder musculature now being dependent relative to the hydrostatic indifference zone.

Furthermore, the estimated FD value of approx. 1006 g/l indicates a mean filtrate PC of 20-24 g/l which is near to the PC of whole-body lymph.

The results are in accordance with previous findings of blood volume changes amounting 5-12 % due to change in body posture of resting humans, occurring within 30-60 min after tilting. In contrast to former assumptions, we found that the fluid shifted between intra- and extravasal compartment is far away from being protein-free, but rather is similar in composition to lymph.

From a methodological point of view, it can be stated that the application of the MOT for frequent determinations on human microsamples, got micro-invasively, is a proved procedure, well suited for the study of influences of gravitational forces, of microgravity and of clinical bedrest, on transvascular dynamics and body fluid distribution.

We have performed preliminary experiments using whole body immersion and exercise. Under μ-g conditions, test protocols including dynamic orthostatic and/or exercise stress should be applied (LBNP, ergometer tests). Experiments of this kind have to rely on low-mass portable density measurement devices which are currently under development, or on sufficient facilities to store ultra-frozen blood samples.

In conclusion, our experiences so far indicate that the method of measuring blood and plasma density is simple and useful for assessment of transvascular fluid shifts due to gravitation and/or μ-g.

REFERENCES

1. Hinghofer-Szalkay, H., et al. 1980. Biomed. Techn. 25:151-157.
2. Hinghofer-Szalkay, H. 1980. Klin. Wochenschr. 58:1147-1154.
3. Kenner, T., et al. 1977. Europ. J. Physiol. 370: 25-29.
4. Kenner, T., et al. 1980. Basic Res. Cardiol. 75: 501-509.

EFFECT OF SUSTAINED G_Z ACCELERATION ON LUNG FLUID BALANCE: AN ULTRASTRUCTURAL STUDY

W. J. Weidner, L. F. Hoffman and D. O. DeFouw

Department of Animal Physiology, University of California, Davis and
Department of Anatomy, College of Medicine and Dentistry
of New Jersey, Newark

In order to examine the effects of sustained acceleration on lung fluid balance, chickens were centrifuged at $+2G_z$ or $+4G_z$ for one hour. At the end of the centrifuge run, the animals were immediately anesthetized with pentobarbital and the lungs were fixed by tracheal instillation at 20 cm H_2O pressure with 2% glutaraldehyde. The fixed lungs were cut into 2 cm thick longitudinal slices and tissue blocks were taken from an upper lung portion, middle lung portion and lower (dependent) lung portion. After post-fixation, thin sections were cut and examined with a Phillips EM 300 electron microscope. Interstitial pulmonary edema was observed in those animals subjected to $4G_z$ acceleration. Micromorphometric data indicate that thickening of the pulmonary interstitial space separating alveolar epithelial cells and capillary endothelium occurred in the $4G_z$ group. Both 2G and 4G acceleration were associated with a decrease in mean capillary diameter. Endothelial vesicular density was progressively reduced in upper and middle lung sections at 2G and 4G, while this parameter was progressively increased in dependent lung sections. Results suggest that pulmonary edema can occur at acceleration intensities below $5G_z$ if centrifuge runs are prolonged.

INTRODUCTION

Positive acceleration (+G) is known to induce many circulatory alterations in animals and man, including redistribution of the circulating blood volume to dependent vascular regions, both in the systemic circulation and in the lung (1).

Pulmonary edema has been proposed as a possible contributor to the development of acceleration-induced alveolar atelectasis; however, it has been suggested that +G is unlikely to lead to edemogenesis at fields of less than $+5G_z$, unless centrifuge runs are prolonged (1). While no macroscopic evidence of pulmonary edema has been reported in response to +G of brief duration, we have recently reported the effects of sustained +G on pulmonary fluid balance (2) and found that exposure to $+4G_z$ for 60 min resulted in an elevated extravascular lung water content in domestic fowl. The present study reports the results of electronmicroscopic investigation of the lungs of domestic fowl exposed to sustained +G and supports our earlier conclusion that pulmonary fluid balance can be altered by prolonged exposure to +G.

METHODS

We used twelve adult domestic fowl of different breeds, ages and sexes, weighing an average of 1.83 kg in this study. Animals were exposed to sustained +G in a hydraulic-driven centrifuge at the Chronic Acceleration Research Unit of the University of California at Davis. During centrifugation, the animals were restrained in a supportive harness to insure support of the neck and head. The acceleration field intensities were calculated to the approximate location of the animal's heart while in the centrifuge carriage.

Animals were accelerated at either $+2G_z$ (N=4) or $+4G_z$ (N=4) for 60 min. Control animals (N=4) were subjected to the same handling and surgical procedures as experimental animals, but were not centrifuged.

At the end of the centrifuge run, the animals were immediately anesthetized with sodium pentobarbital and lungs fixed by tracheal instillation of 2% glutaraldehyde at 20 cm H_2O pressure. The fixed lungs were cut into 2 cm thick longitudinal slices, and tissue blocks were taken from the upper (U) portion, middle (M) portion and the lower (L), most dependent lung portion. After post-fixation, thin sections (60-90 nm) were cut and examined with a Phillips EM 300 electron microscope operating at 60 kV.

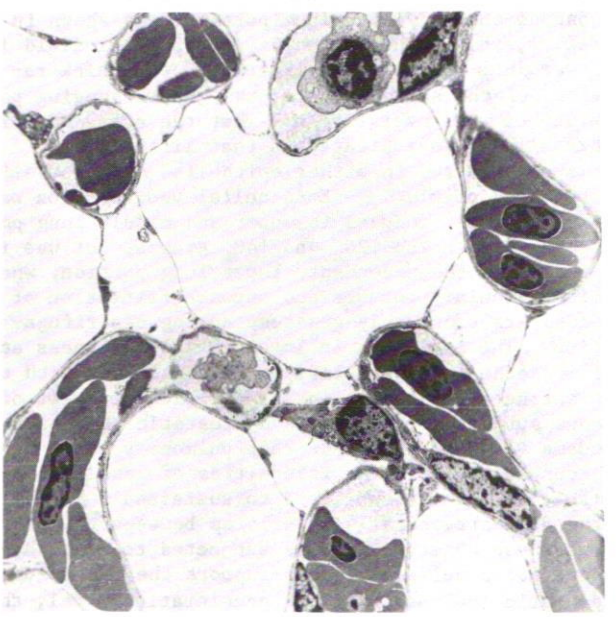


Fig. 1. X6,699, $+4G_z$, lower lung portion, showing fluid accumulation in interstitial space.

TABLE 1. Structural Changes Associated With Sustained $+G_z$ in Lungs of Domestic Fowl

	$\bar{D}$, CAP (μm)[1]	$\bar{D}$, AS (μm)[2]	EP (μm)[3]	INT (μm)[4]	EN (μm)[5]	V_{en}[6]	V_{ep}[7]
CONTROL							
(U)pper	10.3	13.6	0.08	1.2	0.13	27	3
(M)iddle	13.5	18.9	0.08	1.3	0.15	33	4
(L)ower	10.0	11.8	0.07	1.0	0.11	21	6
Mean	11.25	13.0	0.07	1.15	0.13	27	4
$+2G_z$							
U	10.4	16.5	0.06	0.9	0.12	24	3
M	8.9	12.3	0.04	1.3	0.11	28	4
L	9.2	14.2	0.04	1.1	0.11	23	4
Mean	9.50	14.35	0.05	1.10	0.11	25	4
$+4G_z$							
U	7.7	12.5	0.07	1.2	0.12	23	2
M	8.7	13.6	0.08	1.5	0.13	20	4
L	9.3	13.6	0.05	1.8	0.13	26	4
Mean	8.60	13.23	0.06	1.51	0.13	23	3

[1]Ave. diam. capillaries.

[2]Ave. diam. air spaces.

[3]Ave. epithelial thickness.

[4]Ave. interstitial thickness.

[5]Ave. endothelial thickness.

[6,7]Ave. number per unit vol. of endothelial (V_{en}) or epithelial (V_{ep}) vesicles.

RESULTS

Average capillary diameter, but not average air space diameter, was progressively decreased in the $+2G_z$ and $+4G_z$ groups. Sustained acceleration at $+4G_z$ for 60 min was found to produce structural changes associated with interstitial pulmonary edema. Average interstitial thickness was increased in this group of animals, largely as a result of a substantial thickening observed in the most dependent, lower lung portion. As shown in Fig. 1, this accumulation of interstitial fluid in lower lung portions was limited to the thick regions of the septa, sparing the gas exchanging portions of the interstitium. That the edema was extracellular is indicated in that little or no change occurred in either epithelial or endothelial thickness (Table 1). Endothelial vesiculation was progressively reduced in upper and middle lung portions in both the $+2G_z$ and $+4G_z$ groups, but was increased in the dependent, lower lung portion, where microvascular pressure and outward filtration of fluid are presumably greatest during centrifugation. The increases in interstitial thickness and in endothelial vesiculation are consistent with ultrastructural changes in the air-blood barrier of dogs subjected to moderate hydrostatic pulmonary edema (3,4) and indicate that pulmonary edema can occur at acceleration intensities of less than $+5G_z$, in animals subjected to sustained +G. While the ultrastructural similarities between this study and those observed in dogs subjected to acute hydrodynamic pulmonary edema support the use of the domestic fowl as an animal acceleration model, the structural differences between the avian and mammalian lung warrant further study of acceleration-induced pulmonary edema in mammals.

REFERENCES

1. Glaister, D. H. The effects of gravity and acceleration on the lung. p. 127. Agardograph No. 133, Slough, England: Technivision Services.
2. Weidner, W. J. and L. F. Hoffman. The effect of sustained $+G_z$ acceleration on extravascular lung water content in domestic fowl. Aviat. Space Environ. Med. 51:579-582, 1980.
3. DeFouw, D. O. and P. B. Berendsen. Morphological changes in isolated perfused dog lungs after acute hydrostatic edema. Circ. Res. 43:72-81, 1978.
4. Chinard, F. P. The alveolar-capillary barrier: Some data and speculations. Microvasc. Res. 19:1-17, 1980.

VESTIBULAR EFFECTS OF WATER IMMERSION AND CLONIDINE

S. Vesterhauge, A. Månsson, F. Bonde-Petersen, P. Norsk and K. Zilstorff.

Otoneurological Laboratory, E.N.T. dept. 2071, Rigshospitalet and August Krogh Institute, DK-2100 Copenhagen Ø, Denmark.

INTRODUCTION

Changes in cerebral circulation caused by cerebrovascular disease are the most common cause of dizziness. During micro gravity conditions, changes in circulation occur simultaneously with the micro gravity stress on the labyrinth and it is unclear how the circulatory changes modify the vestibular function and how it contributes to the development of space motion sickness. Circulatory changes similar to those occurring during microgravity can be induced by head-out water immersion (WI) and these changes are modified by the administration of Clonidine (CL) (1).

METHODS

Five healthy young male students with normal vestibular pretest volunteered in the study. The effect of CL, 0.3 mg per os, was compared with placebo in a double blind test. The tablet was ingested two hours piror to WI. Circulatory and vestibular parameters were measured immediately before, and after 30 min and 120 min WI, respectively. The subject was seated on a chair in 35 degrees C stirred water.

Vestibular tests: Caloric stimulation was performed binaurally with a closed loop irrigation apparatus, using a 30 degrees C stimulus for 40s. The culmination of slow phase velocity of the nystagmus was calculated with the computer standard technique of our laboratory (2). Further a natural stimulation method was utilized. The subject performed active horizontal sinusoidal head movements guided by a frequency modulated mono sound signal. Two movement patterns were obtained: a stable at 0.5 Hz (for 50 s), and a sweep covering 0.1 to 1.0 Hz (for 100 s). The movements were recorded by an accelerometer and the nystagmus response by standard electro oculographic (EOG) technique. Gain and phase lag of the oculomotor response were calculated by cross power spectral analysis.

Circulatory parameters: Heart rate, blood pressure, cardiac output (CO), and peripheral and splanchnic blood flows were recorded.

RESULTS

Heart rate decreased 10% during WI and was uninfluenced by CL. The systolic as well as diastolic blood pressure was reduced 5-10% during WI and a further 10-15% by CL. WI caused a 20% increase in CO (Fig. 1). Pre-WI CO was reduced 20% by CL. WI also increased CO during CL as shown in Fig. 1.

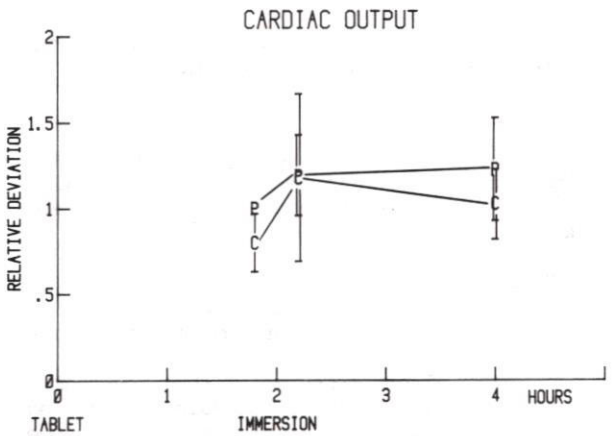


Fig.1 Mean and SD normalized in relation to pre-WI placebo results. P=placebo, C=Clonidine.

WI tends to increase the caloric response (Fig.2). CL inhibits the caloric reponse at control as well as the enhancing effect of WI.

Fig. 3 demonstrates the modification of the phase during active head rotations at 0.5 Hz (stable sinus). The results at 0.1 Hz and 0.2 Hz (sweep) are almost identical. At higher frequencies of the sweep, the power contents of the responses were too weak for safe conclusions. At the three frequencies evaluated, no modification due to WI can be demonstrated, whereas CL significantly increases the phase lag by an average of 78, 42, and 93 degrees at 0.5, 0.2, and 0.1 Hz respectively.

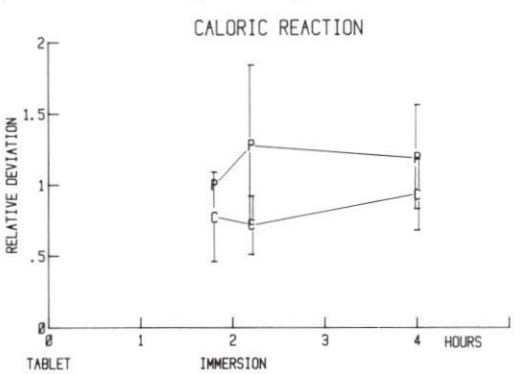


Fig. 2. Mean and SD normalized in relation to pre-WI results. P=placebo , C=Clonidine.

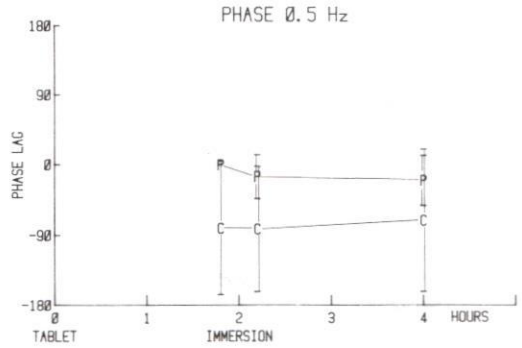


Fig. 3. Mean and SD normalized in relation to pre-WI results. P=placebo, C=Clonidine.

Values calculated for gain were not significantly different from control pre-WI with placebo in any frequency, neither during placebo nor during CL.

DISCUSSION

In vertebro-basilar ischemic patients, no specific pattern of vestibular abnormalities can be demonstrated (3). Hordinsky & Aust (4) demonstrated alterations of the nystagmus frequency following caloric stimulation during WI, but found no significant changes in eye velocity contrary to the present results. Under conditions of alternating hypo- and hyper- G-loads during parabolic flights, it has been demonstrated, that the vestibulo-oculomotor gain varies directly proportionally to the G-load (5), whereas phase relations never have been tested.

During CL, all subjects were influenced by the sedative effects of the drug. Drugs causing sedation tend to inhibit the caloric response (6). The effects on phase deviations have never been described. An enhancement of the caloric response had been demonstrated to be an effect of centrally stimulating drugs (amphetamine and caffeine) (6). This effect might be compared to the WI conditions.

CONCLUSION

1. Circulatory changes induced by simulated weightlessness increase the unphysiologic caloric response, but do not influence the physiological function of the semicircular canals.

2. Clonidine reduces the caloric response and increases the rotatory phase lag probably due to an 'unspecific' central sedation, rather than circulatory changes.

ACKNOWLEDGEMENTS

This study was supported by The Danish Space Board and Boehringer Ingelheim.

REFERENCES

1. Guell et al., (1981) This symposium
2. Vesterhauge, S., Runge, B., Månsson, A. & Zilstorff, K. (1981). Computer analysis of electronystagmographic data. Clin Otolaryngol 6,121-124.
3. Barber, H.O. & Dionne, J. (1971) Vestibular findings in vertebrobasilar ischemia. Ann Otol 80,805-812.
4. Hordinsky, J.R. & Aust, G. (1981) Personal communication
5. Lackner, J.R. & Graybiel, A. (1981) Variations in gravitoinertial force level affect the gain of the vestibulo-ocular reflex: Implications for the etiology of space motion sickness. Aviat Space Environ Med 52, 154-158.
6. Vesterhauge, S & Peitersen, E. (1979) The effects of some drugs on the caloric induced nystagmus. Adv Oto-Rhino-Laryng 25.173-177.

THE EFFECT OF CLONIDINE ON PERIPHERAL VASOMOTOR REACTIONS DURING SIMULATED ZERO GRAVITY

F. Bonde-Petersen, A. Guell, K. Skagen, and O. Henriksen

Laboratory for human physiology, August Krogh Institute, Universitetsparken 13, University of Copenhagen, DK-2100 Copenhagen, Denmark and Department of Neurology, CHU Rangueil, F-31054 Toulouse, France.

INTRODUCTION

The mechanism behind the peripheral vascular reactions during simulated micro gravity (μ-g) has not been extensively studied. The forearm blood flow (FBF) has been shown to increase during 6 hours water immersion (WI) or head down tilted bedrest (BR) (1) but tends to decrease during 20 hours BR (2). Are these changes due to locally or centrally induced changes during the simulated μ-g? It was, therefore, thought of interest to study the influence of long term BR upon the venous compliance (VC) and the vasocontrictor response in the arterioles during local increases in hydrostatic pressure (3) and during tilt. BR was also combined with Clonidine (CL) medication. CL stimulates the CNS cardiovascular depressor center and stimulates the peripheral alpha-receptors.

METHODS

Three normal subjects were treated with CL and three were controls during a 7 days BR study as described by Guell et al. (4).

Skin blood flow was studied by the local 133-Xe clearance, and the relative to control skin vascular resistance calculated. Injections of 100-150 μCi of 133-Xe were performed at the dorsal wrist and ankle. Resistance changes during +45 degrees head up tilt, and local lowering of arm and leg during horizontal bedrest, respectively, were compared with horizontal control. In this way the local veno-arteriolar constrictor reflex (3) and the combination of this with increased sympathetic nervous activity (SNA) could be evaluated (5).

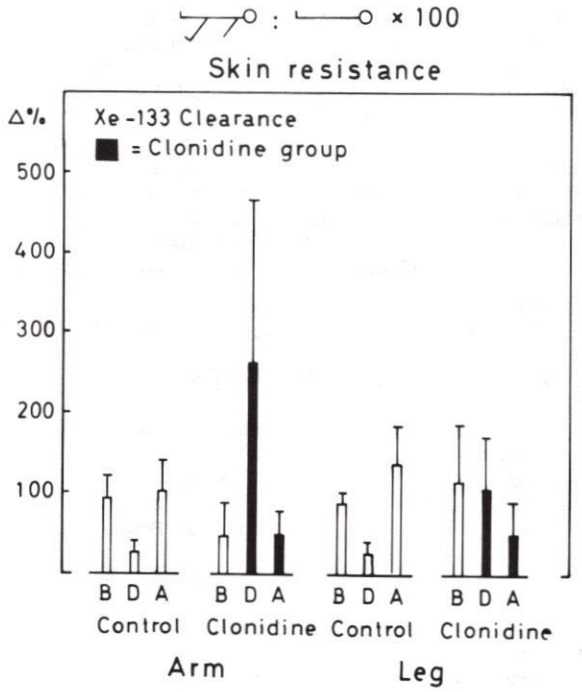


Fig. 1

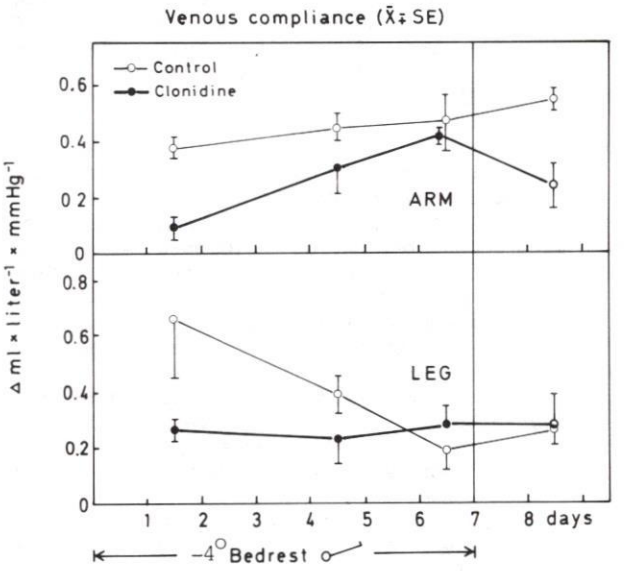


Fig.2

FBF, leg blood flow (LBF), and VC were measured by venous occlusion plethysmography using an air-in-rubber cuff sytem at 3 mmHg (6).

Due to the small number of subjects observed, no statistical calculations were performed except for mean values and SE.

RESULTS

The arteriolar constrictor reflex activated during increased local hydrostatic pressure decreased in arms and legs during BR, but increased by a factor of 2.9 and 1.7 respectively during BR+CL. After BR the reflex was back to normal in the control group but somewhat weakend in the group treated with CL (Fig. 1). This increase in arteriolar tone was most pronounced in the arms compared with the legs. In the legs, CL prevented a fall in the arteriolar reflex during BR. If SNA was increased by +45 degrees tilt (a baroreceptor reflex) the same pattern was observed as by locally increased hydrostatic pressure. However, tilt was only performed before and after

BR, and, therefore, this comparison is incomplete.

During BR, FBF and LBF did not change (Fig. 2). This trend was not changed by CL. However, 48 hours after medication was discontinued, and 36 hours after termination of the BR period, resting FBF and LBF doubled in the group treated by CL.

The VC was definitely lower in both arms and legs of the CL treated group during the second day of BR (Fig. 3). In the arms VC was slightly increasing during BR in the controls but decreased by a factor of ca 4 in the legs over 7 days. However, arm-VC increased during BR in the CL-group. 36 hours after BR VC had still not recovered. In the legs VC was lowered by BR+CL; the control group showed an adaptation of VC toward the treated group. When BR was discontinued, this did not affect the 36 hours value after BR.

DISCUSSION

BR per se changed both the arteriolar and venous tone during BR. The local veno-arteriolar reflex decreased equally in arms and legs, and recovered soon after BR. CL evidently stimulated alpha receptors peripherally, so that the reflex was either increased (arms) or the fall in reactivity prevented (legs) (Fig. 1). Tilting did not modify this response, but results during BR are lacking.

FBF and LBF showed no change during BR, but recovery values after CL were increased by 100% at 36 hours, possibly due to a decrease in arteriolar tone after withdrawal of CL-medication, an "over shoot" phenomenon.

Forearm VC, which was decreased by CL compared to the controls, adapted slowly during BR to the control values, which were also slightly increasing, possibly due to the increased hydrostatic pressure in the arms during BR. The opposite effect was seen in the legs, where VC,

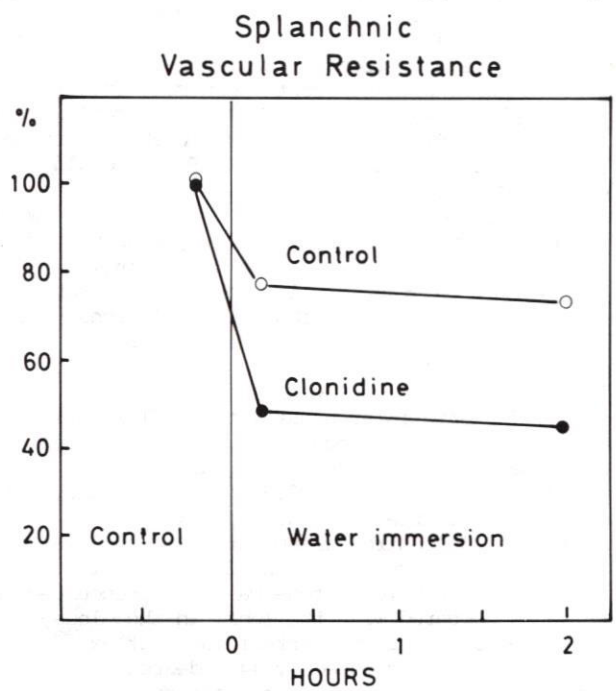


Fig.4

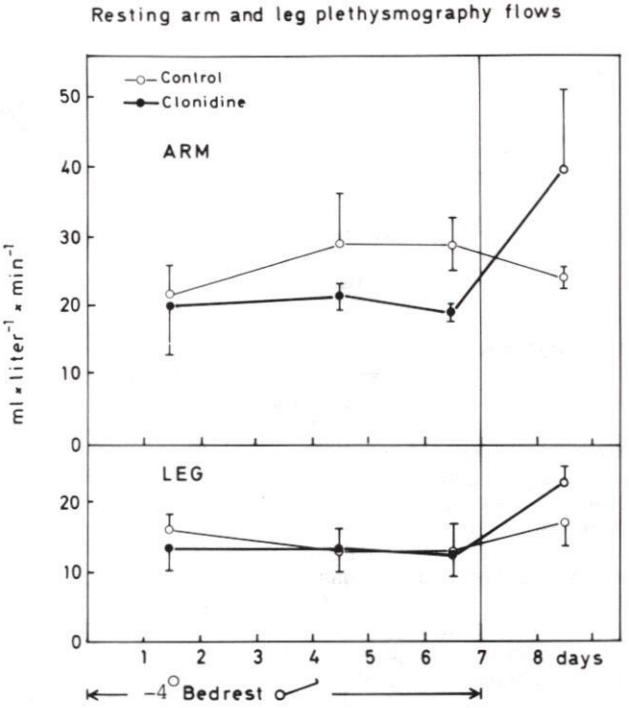


Fig.3

which was decreased due to CL, did not adapt, while a steady decrease was observed in the controls (Fig 3), possibly due to the decreased hydrostatic pressure in the legs during BR. This explains the "craw legs" of the astronauts as being due, not only to loss of interstitial fluid volume but also due to an increased venous tone, thus adding to the fluid shift during u-g.

The present results add to the understanding of the peripheral effects of CL, which evidently acts as a peripheral alpha-stimulator. The main effect of CL is as a central SNA depressor. This effect can be studied in Fig. 4, where the splanchnic vascular resistance studied with the indocyanine method (7) during WI and CL (8). WI per se depresses SNA, and an additive effect of WI and CL shows a further reduction of SVR down to 50% of control values. This effect counteracts the increased total peripheral resistance which otherwise would be a result of an increased alpha stimulation, so that fall in mean arterial pressure was observed.

REFERENCES

(1) Bonde-Petersen, F.et al., Physiologist 23:S7-S10.1980
(2) Norsk, P. et al., Physiologist. This Suppl. 1981.
(3) Henriksen, O., Acta Physiol Scand Suppl. 450.1977.
(4) Guell, A. et al., Physiologist. This Suppl.1981.
(5) Skagen, K. et al., Acta Physiol Scand 111:113-120.1980.
(6) Dohn, K. et al., Rep Steno Mem Hosp 6:141.1956.
(7) Rowell, L.B., et al., J Clin Invest 43:1677-1690.1964.
(8) Bonde-Petersen, F. et al., Unpublished observation 1981.

CARDIOVASCULAR EFFECTS OF CLONIDINE DURING 20 HOURS HEAD DOWN TILT (-5°)

P. Norsk, F. Bonde-Petersen and J. Warberg
August Krogh Institute, and Institute of Medical Physiology C, University of Copenhagen, Denmark

INTRODUCTION.

Bedrest at -5 degrees head down tilt (BR), has been used for simulation of a microgravity (u-g) environment on the human cardiovascular system (1). BR and u-g produce a central fluid shift resulting in a decreased plasmavolume. This is mediated by activation of low pressure baroreceptors in the intrathoracic region causing reduced anti diuretic hormone (ADH), and renin-aldosterone plasma levels (2). Orthostatic tolerance and exercise capacity is reduced after tilt and space missions (1,3,4,5).

Clonidine (CLO) is an antihypertensive drug that lowers blood pressure and decreases sodium excretion (6). Guell et al. (7) showed that CLO during 7 days BR (-4 degrees) prevented hypovolemia from the second day.

We investigated the cardiovascular effects of CLO during 20 h BR and the effects of CLO upon exercise capacity and orthostatic tolerance pre- and post-BR.

METHODS

Five young healthy males were investigated twice with and without CLO according to the same protocol during 20 h BR.

Arterial pressures were measured by a sphygmomanometer, heart rate (HR) and ECG monitored by a scope, forearm blood flow (FBF) and specific venous compliance (SVC) was measured by venous occlusion plethysmography, cardiac output (CO) and Lung tissue volume (LTV) by acetylene- and argon rebreathing, ADH plasma level by radio immuno assay, and relative change in plasma volume (PV) by changes in hematocrit and hemoglobin. Orthostatic tolerance was measured pre- and post-BR by Lower Body Negative Pressure (LBNP) following the Skylab protocol (1,5). LBNP-tolerance was indicated by the subject at the sudden occurrence of nausea. During LBNP HR, and FBF were measured. Submaximal (150 Watts) and maximal exercise capacity (stepwise 30 Watts increments every 3 min.) were performed pre- and post BR on a bicycle ergometer while HR and oxygen uptake (VO2, Douglas method) were measured.

The subjects received an oral dose of 0.15 mgx3 daily starting 24 hours before the eksperiment.

RESULTS

Mean arterial pressure (MAP) in the supine position before BR did not change during CLO compared to control without CLO (CTR) (85/5 (MEAN/SE) and 84/3 mmHg respectively) (Fig. 1). CLO lowered HR from 69/4 to 58/2 bpm ($P<0.02$) and CO from 7.53/0.65 to 6.54/0.21 l/min; stroke volume (SV) increased from 108/7 to 113/8 ml. BR per se increased MAP from 84/3 to 94/4 mmHg

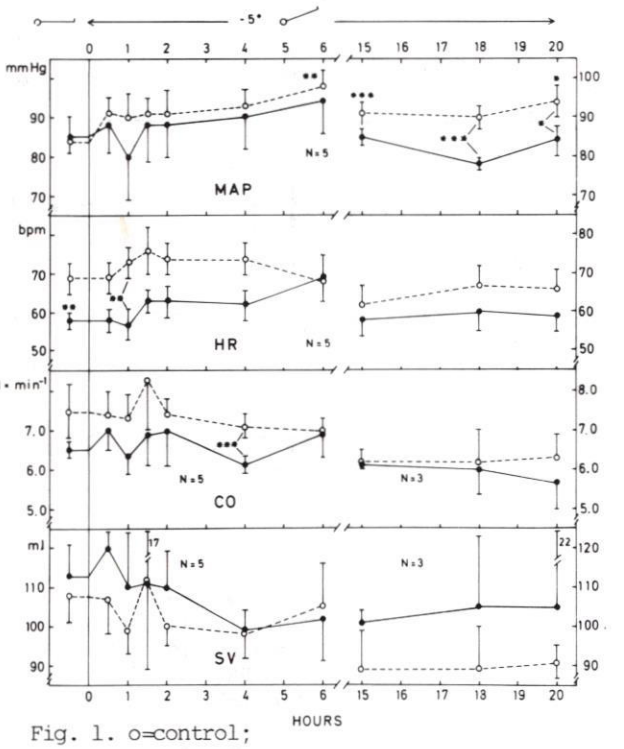


Fig. 1. o=control; ●=clonidine.

($P<0.05$). CLO counteracted this effect. During BR HR, and CO were lower during CLO than in the CTR situation.

CLO increased forearm vascular resistance (FVR) as well as total peripheral resistance (TPR) pre-BR in the supine resting position (Fig. 2). During CTR-BR FVR increased from 95/12x10 PRU to 201/36x10 PRU; TPR increased from 0.64/0,05 to 0.89/0.02 PRU. CLO had no further significant influence on FVR during BR even if TPR tended to increase from 0.71/0.05 to 0.89/0.1 PRU.

CLO decreased SVC pretilt in the supine position and correspondingly an increase in LTV was seen (Fig. 3). During BR SVC was unchanged in the CTR situation while CLO+BR increased this parameter. LTV was increased within the first hour during CLO+BR (from 0.98/0.06 to 1.05/0.13 l) but later decreased (0.79/0.14 after 20h BR). BR increased LTV the first 6 hours from 0.89/0.05 to 1.00/0.08 ($P<0.01$) but LTV decreased during the following 14 hours to 0.79/0.08. PV decreased due to BR by 12.8/2.8% after 20 h BR (significantly after 15 h BR, $P<0.01$) while CLO+BR decreased PV more rapidly

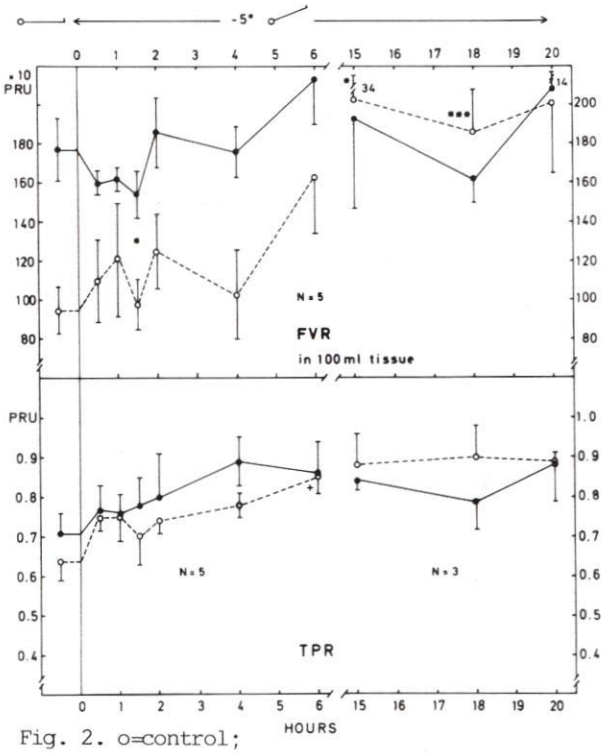


Fig. 2. o=control;
●=clonidine.

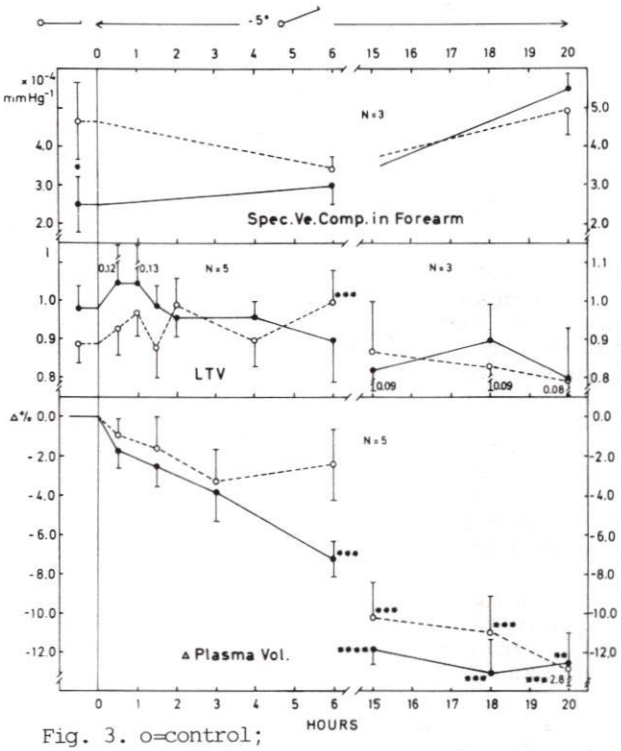


Fig. 3. o=control;
●=clonidine.

but to the same extent. LBNP tolerance was decreased due to BR (9.0/1.9 min pre-BR to 5.2/0.5 min post-BR). CLO and BR did not change this effect (9.6/2.2 min pre-BR and 4.5/1.4 min post-BR). HR during -30 mmHg LBNP was increased after CTR-BR (from 107/9 bpm pre- to 120/6 bpm post-BR) while BR+CLO did not modify HR at -30 mmHg (93/6 and 93/12 mmHg, respectively). HR increased after CTR-BR during submaximal exercise (134/5 bpm pre-BR and 149/9 bpm post-BR); BR and CLO also increased HR at submaximal exercise (127/6 bpm pre-BR and 139/12 bpm post-BR) but at a lower level. ADH did not change significantly with time during CTR-BR (3.6/0.7 pg/l pre-BR and 2.6/0.2 post-BR) or during CLO+BR (4.2/1.5 pg/l pre-BR and 2.8/0.3 post-BR). CLO did not affect ADH plasma levels.

DISCUSSION.

At the high pressure (arterial) side of the circulation BR augmented TPR, FVR, and MAP, probably due to an increased peripheral vasoconstrictor tonus. BR induced a higher level of stress as HR was increased during LBNP and submaximal exercise post-BR. The same effect was found after the Skylab and the Apollo missions (3,4,5). CLO counteracted the increase in MAP during BR due to a decrease in CO and HR. This is accomplished by the stimulation of the alpha-adrenergic receptors in the depressor area of medulla oblongata (8). CLO increased FVR and TPR due to stimulation of the peripheral alpha receptors (8). The post-BR tachycardia during LPNP and exercise stress is reduced by CLO due to the central nervous effect of the drug.

At the low pressure (venous) side of the circulation BR per se did not change SVC in the forearm. This parameter is reduced by CLO pre-BR. This concurs with the concommittant increased LTV. BR+CLO reduced LTV and PV more rapidly than BR alone, probably because of the increased intrathoracic bloodvolume induced by CLO. The ADH plasma level was not changed in the 5 subjects due to BR or to BR+CLO.

In conclusion: The stress induced by 20 hours BR is counteracted by the central depressor effect of CLO on the heart. The peripheral effect of CLO increasing FVR, venous tone, and LTV enhances the adaptation to the u-g condition.

REFERENCES

(1) Nixon,J.V. et al. J Appl Physiol: Respirat Environ Exercise Physiol 46:541-548,1979.
(2) Gauer,O.H. et al. In: International Review of Physiology. Cardiovascular Physiology II. 9:145-190,1976.
(3) Rummel,J.A. et al. Aviat Space Environ Med 47:1056-1060,1976
(4) Rummel,J.A. et al. Aviat Space Environ Med 46:679-683,1975.
(5) Johnson,R.L. et al. Aviat Space Environ Med 47:347-353,1976.
(6) Onesti,G. et al. Circulation 219-228,1969.
(7) Guell et al. Physiologist. This Suppl. 1981.
(8) Houston,M.C. Progress in Cardiovascular Diseases 337-350,1981.

CARDIAC AND CEREBRAL VASCULAR ADAPTATION TO GRAVITATIONAL STRESSES IN MAN

ALDO BELLUSCHI

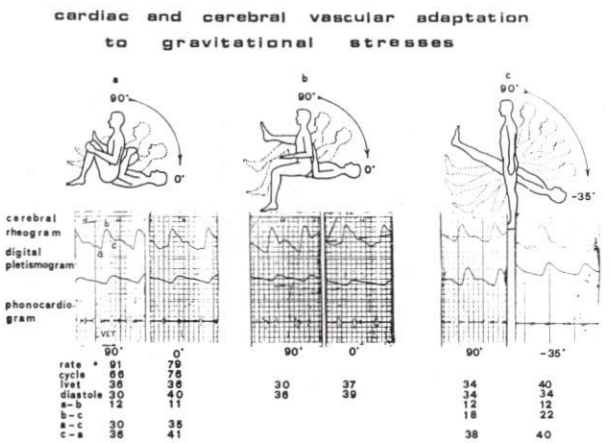
OSPEDALE MAGGIORE "CA' GRANDA" - 20100 MILAN, ITALY

General notions of the influence of the gravity force on general and cerebral blood flow are already known;on the contrary the ways and processes by which the human organism reacts to it, particularly in the encephalic area,have not been specified yet Many are the mechanisms that take part in the control of the cerebral blood flow, changing in their reciprocal relations, different from person to person, so that it appears impossible to draw absolute conclusions or to state laws. We can however investigate the reactions of the human body in different situations to the gravity force.

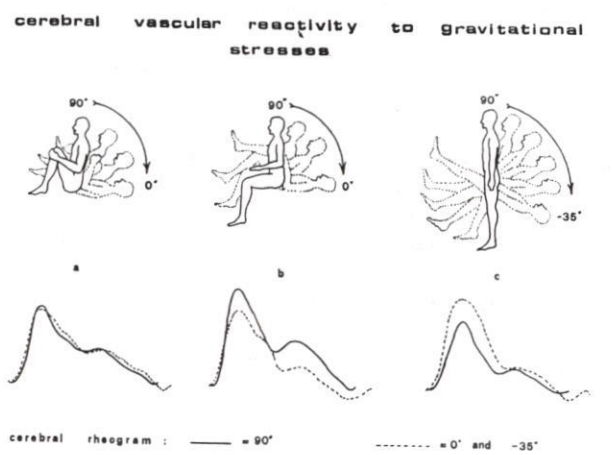
A polygraphic method, consisting of cerebral rheogram (REG), digital plethysmography and phonocardiogram (PCG) was used to analyse the behaviour of the cerebral vascular resistances and that of the cardiac output. The REG wave must be regarded as the linear recording of a tridimentional phenomenon, i.e. the variations that the stroke volume undergoes passing through the arterial and venous vessels of a cerebral area, for instance the left Sylvian artery area. The digital plethysmogram is assumed to compare the general peripheral pulsation to the cerebral one. By the PCG we know the systolic and diastolic time intervals. By this method which proved to be safe, non-invasive, easy to perform and of highly informative value, recordings of cardiac and vascular reactions were performed in healthy subjects undergoing passive changes of posture in three different moments (fig.1):

a.- from vertical (90°) to horizontal (0°) with the body in a crouched posture.
b.- from vertical (90°) to horizontal (0°) with the body in a sitting posture.
c.- rotation of the body, stretched, from upright (90°) to head-down (-35°)

One of the main effects of the rotation of the body is the shift of the blood mass towards the sloping parts of the body. This displacement occurs in different ways in our examples, both for quantity and speed: little in the first, remarkable in the second and heavy in the third.



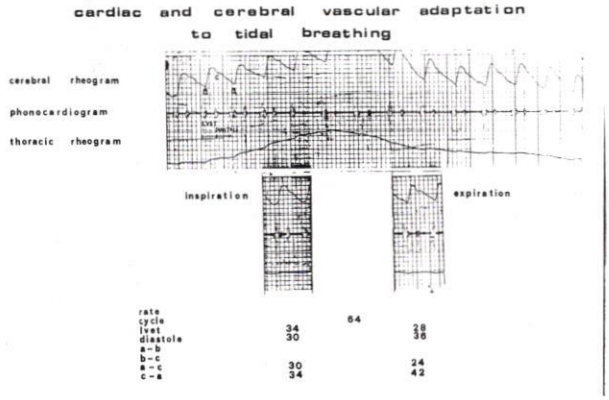
In the first case (fig.1 a) the rotation of the heart-brain axis determines a relative rise in the diastolic time interval while the systolic output is unchanged. The cerebral pulse shows little changes essentially consisting in a slight delay of the venous outflow. By superimposing the two waves the changes are evident.(fig.2 a)



In the second case (fig.1 b) the diastolic time interval rises from 0.36" to 0.39" while the systolic time interval rises from 0.30" to 0.39" from which we infer an increase in the cardiac output. The REG wave shows a reduction in amplitude and a delay in the venous outflow. The superimposing of the waves gives evidence of these changes. (fig.2 b).

In the third case (fig.1 c), which should be regarded as an excess model, we find an elective increase of the systolic time interval from 0.34" to 0.40" and a noticeable variation of the cerebral pulse; the REG wave is higher, greater in amplitude and delayed in the venous segment. (fig.2 c).

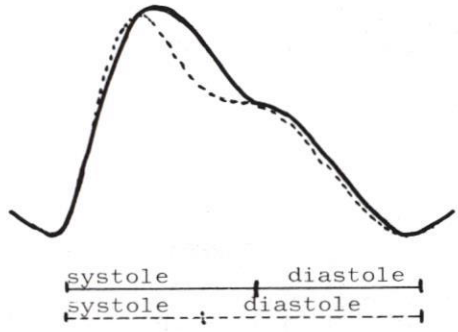
During this investigation the influence of the tidal breathing has come out. (fig.3).



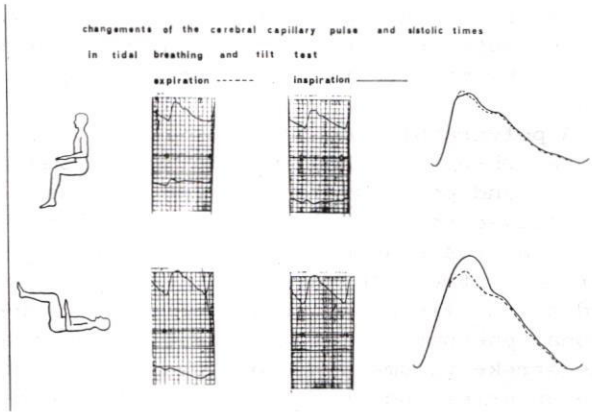
A careful observation of the REG records confirms that the cerebral pulse shows different and constant characteristic depending on the in- and expiratory phases. The morphology of the apex of the REG wave is rounded in the expiratory phase and sharp in the expiratory one: this finding suggests different modes of pulsation of the cerebral capillary artero-venous network. Tidal breathing applies constant influence on the cerebral blood flow, specially on the venous outflow determining a rhythmical dilatation and constriction of the artero-venous capillary vessels (fig.4)

To illustrate how the single influence of gravitational vector and tidal breathing integrate, we report cardiac and cerebral vascular reactions to changes of posture and to in- and expiratory phases. (fig.5)

CHANGES OF THE CEREBRAL CAPILLARY PULSE AND SYSTOLIC TIMES IN TIDAL BREATHING



——— inspiration
----- expiration



In conclusion, we can say that:

a.- the influence of the gravitational vector, which is constant in its direction, changes in the different postures, in the arterial and venous network, in the brain and in the peripheral area;

b.- The gravitational vector is a stimulus for the regulation of the arterial tone

C.- The gravitational vector, associated with the thorax bellows, is a coefficient of the venous outflow and as such a stimulus to the cerebral capillary vascular reactions. During inspiration the venous outflow gets quicker,within the skull it induces a depression which determines a capillaro-venous vasodilatation.

RESPONSE OF RAT BODY COMPOSITION TO SIMULTANEOUS EXERCISE AND CENTRIFUGATION AT 3.14g

Grover C. Pitts and Jiro Oyama
Department of Physiology, School of Medicine, University of Virginia, Charlottesville, VA 22908, USA and Biomedical Research Div., Ames Research Center, Moffett Field, CA 94035, USA.

INTRODUCTION-Skeletal muscle required to contract against markedly increased physical loads may show changes including increased mass (1). Then chronically centrifuged rats, being exposed to such increased loads, should show increases in mass of muscle and possibly other body components. In fact, mass of the total body musculature decreases in centrifuged rats (6). If this decrease reflects the net influence of opposing factors, the effect of the load factor might nevertheless be demonstrated statistically. In any case, the load effect of centrifugation should be clearest when comparing sedentary and active groups since physical activity exposes the major postural and locomotor muscles to the increased load of centrifugation. The present study is designed to test these possibilities.

METHODS-The 2 levels of each experimental variable (acceleration, activity and diet) follow.

ACCELERATION:	1g (3.14g)			
ACTIVITY:	Restrained		Exercised	
DIET:	Chow	HiFat	Chow	HiFat
Group Symbols:	1gRC	1gRF	1gEC	1gEF

The plan at 3.14g is identical. There were 6 male Sprague-Dawley rats, 9 weeks of age (one per cage) in each of the 8 experimental groups which were matched in initial mean body masses. The groups at 3.14g were chronically centrifuged as described previously (6) for 12 days which allowed them to achieve a new steady state of live mass. The 1g groups were similarly caged and treated at terrestrial gravity.

The restrained rats were in cages ∿2x body volume and the exercised ones lived in running wheels (Wahmann Mfg. Co., Baltimore, MD) propelled by their ad lib activity.

The diet groups were provided ad lib with either a pelleted commercial chow (Wayne) or a custom made paste diet with 60% fat described previously (5). Rats consume 50-60% more calories as the high fat diet than as chow, both at 1g and at 3.18g (7).

Dissection of the decapitated rats has been described (3). After recording fresh masses of individual organs the components are recombined into 3 categories, (carcass, skin and viscera) which are treated as units during freeze drying and fat extraction. Net body mass is live mass less masses of fur, gut content and bladder content. Carcass (skinned eviscerated body with brain removed) closely approximates the musculo-skeletal system. Changes observed in the carcass are probably equally descriptive of muscle and bone since the fat-free carcass, as prepared here, appears fairly consistent at 88% muscle and 12% bone (unpublished). Viscera is a pool of all abdominal, thoracic and retroperitoneal viscera with brain added.

The effect of the three experimental variables on each body composition parameter were evaluated statistically by 3-way analysis of variance (9).

RESULTS-During the first 2-6 days of centrifugation live body mass of centrifuged groups dropped to new steady states at 80-85% of the respective 1g groups, a response similar to earlier findings (6,7). In groups exercising during centrifugation wheel-running dropped to nearly zero by day 3, then rapidly recovered. The exercising group means (meters/24 hrs±SE) calculated for centrifugation days 4-12 were: 1gEC - 4803±791, 1gEF - 4789±650, 3gEC - 191±33, and 3gEF - 422±54. The restrained rats, always in precisely the same orientation, probably did not turn around within their cages.

Body composition results are given in Tables 1 and 2. Statistically significant effects of the individual variables (there were no interactions) are summarized in column 6, Table 2. The tabulated parameters are listed in a progressive manner. Thus an observed change in the first, net body mass, may involve changes in any combination of fat, fat-free solids and water. A change in the next parameter, fat-free mass demonstrates persistence of the change after fat has been removed. A change in fat-free dry mass indicates that tissue solids participated in the change and finally a change in mass of water indicates participation of that fluid in the change. The fresh organs were not individually subjected to similar chemical analyses. We see that the acceleration variable changed 18 of the 20 measurements, all but one with $P<.01$. In each case groups at 3.14g < those at 1g as reported previously (6). The diet variable was more limited than acceleration in its effects. Pooled viscera of groups on chow > those on high fat diet. Values for fat-free mass, fat-free dry mass, and water content suggest that both solids and water contributed to this difference. Also chow groups > high fat groups in fresh masses of liver and kidneys. Finally the activity variable was associated with the following changes, most of them at the $P<.05$ level. Exercised groups < restrained groups in net body mass, fat-free body mass and fat-free dry body mass. Body water content did not contribute and this reduction is ascribable largely to losses in solids. Exercise groups < restrained groups in fat-free, fat-free dry and water masses of skin, indicating that both solids and water contributed to this difference. Finally, exercised groups < restrained groups in fat-free dry masses of carcass and pooled viscera and in fresh masses of liver and kidneys.

Although not included in the tables, both masses and percentages of fat in the total body, carcass, viscera and skin changed in response to each experimental variable (with some interactions between variables), confirming the extreme lability in body fatness (4,5). By contrast, water as a percentage of fat-free body, carcass, viscera and skin showed no changes corroborating early reports of constancy (2). In the eight groups water in the fat-free body ranged from 72.36 to 72.65%.

DISCUSSION-The hypothesis that increased physical

load during chronic centrifugation contributes to body composition changes observed in centrifuged rats is now doubtful. Considering fat-free and fat-free dry masses of the total body and the carcass we see that centrifugation combined with either wheel-running or restraint reduced these masses to ~85% of the respective 1g values, i.e., the same result with either sedentary rats or rats running several hundred meters/day. If an effect of centrifugation-induced load were present but hidden by opposing factors, analysis of variance should reveal it as an interaction between acceleration and other variables but there were no such interactions. Possibly centrifugation influences body composition by direct effects on metabolism, as hypothesized (8), which are independent of the loading mechanism.

In general the tables reemphasize the prevading influence of chronic centrifugation (changing 18 of the 20 measurements) after only 12 days exposure.

REFERENCES- 1.Astrand, P.-O. and K.Rodahl. Textbook of Work Physiology. New York:McGraw-Hill Book Co., 2nd ed.1977,p.414. 2.Pace, N. and E.N.Rathbun. J.Biol.Chem 158:685,1945. 3.Pace, N., D.F.Rahlmann and A.H.Smith. Report EPL 79-1, Environmental Physiology Laboratory, Univ. of California, Berkeley,1979. 4.Pitts, G.C. Metabolism 27:469,1978. 5.Pitts, G.C. and L.S.Bull. Am.J.Physiol. 232:R38-R44,1977. 6.Pitts, G.C., L.S.Bull and J.Oyama. Am.J.Physiol. 223:1044,1972. 7.Pitts, G.C., L.S.Bull and J.Oyama. Am.J.Physiol. 228:714,1975. 8.Smith, A.H. and R.R.Burton. The Physiologist 23:Suppl.,Dec.1980. 9.Sokal, R.R. and F.J. Rohlf. Biometry. San Fransisco:W.H.Freeman and Company. 1969.

Table 1. Body Composition at 1G. Group mean masses ± SE.

Components	Exercised/Chow	Exercised/HiFat	Restrained/Chow	Restrained/HiFat
Net Body	302±12	290±18	325±6	338±8
Fat free: Body	285±11	261±16	293±6	282±8
Carcass	181±7	169±10	188±4	179±6
Viscera	35.6±1.4	34.0±2.1	40.3±1.3	37.8±1.2
Skin	47.5±2.8	42.0±2.6	48.4±1.9	47.2±1.0
Fat free dry: Body	78.0±2.4	71.8±3.8	80.2±1.8	77.5±1.7
Carcass	49.0±1.6	46.0±2.6	51.4±0.9	48.9±1.4
Viscera	8.36±.33	7.22±.42	8.85±.29	8.34±.27
Skin	14.0±.7	12.5±.8	14.0±.8	13.8±.2
Water in: Body	207±9	190±12	213±5	205±6
Carcass	132±5	123±8	136±3	130±4
Viscera	30.3±1.1	26.8±1.7	31.5±1.0	29.5±1.0
Skin	33.5±2.1	29.5±1.8	34.4±1.3	33.4±0.8
Fresh Organs:				
Liver	12.6±.7	11.1±.7	13.9±.6	13.5±.8
Genitalia	8.00±1.3	8.04±.70	7.77±.94	8.31±.37
Kidneys	2.59±.09	2.28±.13	2.68±.14	2.53±.07
Brain	1.63±.07	1.53±.14	1.63±.08	1.73±.10
Lungs & Trachea	2.27±.12	2.14±.10	2.28±.10	2.37±.06
Heart	1.21±.06	1.12±.07	1.24±.10	1.29±.05
Adrenals	.069±.006	.062±.002	.065±.006	0.48±.009

Table 2. Body composition of groups at 3.14g. Group mean masses ± SE.

Components	Exercised/Chow	Exercised/HiFat	Restrained/Chow	Restrained/HiFat	Significance
Net Body	240±6	247±7	266±5	267±9	A,C
Fat free: Body	232±5	230±7	251±4	242±7	A,c
Carcass	148±4	151±3	160±2	155±4	A
Viscera	34.4±1.7	30.0±1.4	35.3±0.5	31.0±0.9	A,B
Skin	34.5±1.2	34.9±1.7	39.1±1.3	37.9±1.7	A,c
Fat free dry: Body	62.9±1.3	64.4±1.4	69.1±0.8	66.8±1.8	A,C
Carcass	41.0±1.0	41.3±0.8	43.9±0.7	42.7±1.2	A,c
Viscera	6.90±.16	6.29±.29	7.70±.23	6.22±.46	A,B,c
Skin	10.1±.3	10.7±.4	11.8±.4	11.8±.6	A,c
Water in: Body	167±5	170±4	182±3	175±5	A
Carcass	107±3	110±2	116±2	112±3	A
Viscera	27.5±1.6	23.7±1.1	27.6±0.4	24.8±0.6	A,B
Skin	24.4±1.0	24.3±1.3	27.3±1.0	26.2±1.1	A,c
Fresh Organs:					
Liver	10.9±.4	9.0±.5	11.8±.3	9.7±.3	A,B,C
Genitalia	5.91±.32	7.07±.32	6.93±.10	6.84±.18	A
Kidneys	2.20±.07	2.14±.08	2.42±.06	2.29±.06	A,b,c
Brain	1.45±.08	1.61±.07	1.57±.13	1.54±.04	
Lungs & Trachea	1.83±.08	2.22±.12	2.14±.06	2.05±.12	a
Heart	.90±.03	.98±.04	1.11±.06	1.11±.04	A,C
Adrenals	.064±.003	.067±.004	.074±.005	.073±.009	

Statistically significant results of the individual components, (3-way analysis of variance calculated on data of both tables) are indicated in column 6. A($P<.01$) and a ($.01<P<.05$) indicate signficant effects of acceleration, B (b) of diet and C (c) of activity.

EVIDENCE FOR ARRESTED BONE FORMATION DURING SPACEFLIGHT

R.T. Turner, J.D. Bobyn, P. Duvall, E.R. Morey,
D.J. Baylink and M. Spector

VA Medical Center, Charleston, SC 29403; Medical University of South Carolina, Charleston, SC 29425; NASA-Ames Research Center, Moffett Field, CA 94035; and VA Medical Center, Tacoma, WA 98493.

INTRODUCTION

Bone formation is reduced in the long bones of growing rats during spaceflight (1,2). This defect is quickly corrected upon termination of the flight (1,2). The cause of this reversible inhibition is not known but is probably directly associated with reduced mechanical loading of the skeleton during the near weightlessness of spaceflight. To address the question of whether the bone formed in space is unusual we studied the morphology of bone made at the tibial diaphysis of rats prior to, during, and following spaceflight. We report here evidence of arrest lines in the bone formed in space suggesting that bone formation ceases along portions of the periosteum during spaceflight.

METHODS

Two to three month old rats were flown on 18.5 - 19.5 day orbital spaceflights aboard Cosmos 782, 936, and 1129 biological satellites. Animal care, preflight and postflight activities were performed by the Soviets as described previously (1,2). A flight control group was maintained for each experiment aboard an identical earth based spacecraft and exposed, as closely as possible, to flight conditions; including acceleration, noise, shock and vibration of launch and reentry. The rats received tetracycline prior to spaceflight and 3-6 days following the end of the flight. The rats used in these studies were sacrificed at 25 (Cosmos 782 and 936) or 29 days (Cosmos 1129) postflight. Ground sections were prepared from the left tibia at the tibia-fibula junction (3). The sections were microradiographed (4), and then viewed with visible light, UV and scanning electron microscopy.

RESULTS

Arrest lines were detected near the periosteal surface of tibiae from rats flown in space and not sacrificed until 25-29 days following recovery (Figure 1). Although arrest lines appear to be a normal occurrence in aging rats their formation is greatly accentuated by spaceflight. Also, arrest lines are found in tibiae from suspended rats, suspension being a ground based model similar to spaceflight in that the hind limbs are mechanically unloaded (5). Since suspension as well as spaceflight results in decreased bone formation (1,2,5) it seems likely

Table 1

Properties of arrest lines.

Conditions in which accentuated	1. Spaceflight 2. Suspension 3. Aging
Location during spaceflight and suspension	1. At or near 2nd tetracycline label
Physical Properties	1. Density less than surrounding bone matrix 2. Fractures transmitted along length 3. No acid phosphatase activity

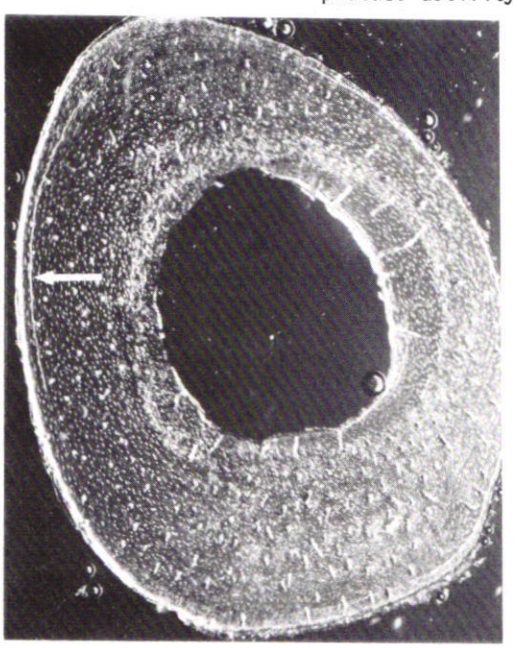


FIGURE 1. A representative ground section from the tibia of a rat flown aboard Cosmos 1129. The lighting is oblique. The arrow points to a prominent arrest line.

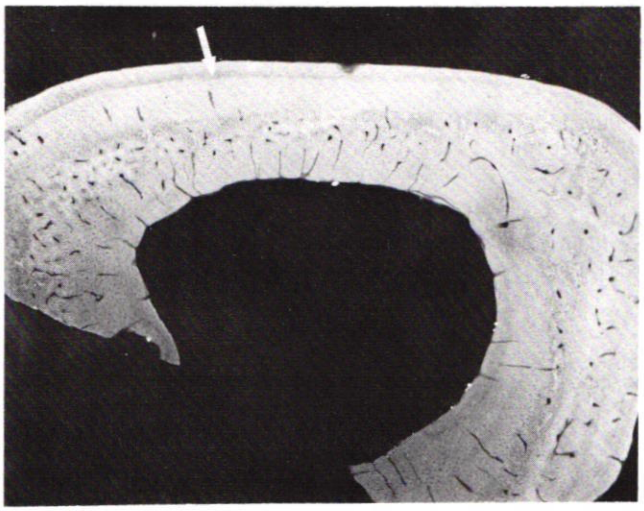


FIGURE 2. A representative microradiograph of a ground section from the tibia of a rat flown aboard Cosmos 1129. The arrow points to an arrest line. Note that the arrest line is darker than the surrounding bone matrix indicating that it is less dense than the matrix.

that the arrest line is a manifestation of this inhibition.

Arrest lines are believed to occur when bone formation ceases along a front and is then reinitiated at a later point in time. Some properties of arrest lines are summarized in Table 1. Unlike a cement line arrest lines form a smooth boundry and do not stain for acid phosphatase activity. Thus, osteoclastic resorption does not occur between cessation of bone formation and its later reinitiation. The arrest line associated with spaceflight is located at the position of the second tetracycline label. As a result, it is not visible in animals sacrificed immediately following spaceflight. These observations imply that bone formation halts along portions of the periosteal surface and remains halted at those sites until the flight is terminated at which point formation is reinitiated. Arrest lines would not be present if the bone formation rate had merely decreased to a reduced level.

When visualized by microradiography (Figure 2) it is clear that arrest lines are less mineralized than the surrounding bone matrix. Also, when viewed by scanning electron microscopy it appears that bone fractures more readily at the site of an arrest line. These observations suggest that arrest lines are a zone of weakness and their formation may result in decreased bone strength in spite of normalization of bone formation following flight (6).

Bone formation is nonuniform along the circumference of the tibial diaphysis in two month old rats and is related to mechanical stress. A reduction in mechanical loading is a likely explanation for decreased bone formation during spaceflight (7). The occurrence, location, and morphology of arrest lines suggest that they are a visible result of the phenomenon of arrested bone formation. It remains to be determined whether there are sites where bone formation proceeds normally during spaceflight.

REFERENCES

1. Morey, E.R., and D.J. Baylink. Inhibition of bone formation during spaceflight. Science 201: 1138-1141, 1978.

2. Turner, R.T., E.R. Morey, C. Liu, and D.J. Baylink. Altered bone turnover during spaceflight. The Physiologist 22: 573-574, 1979.

3. Baylink, D.J., M. Stauffer, J. Wergedal, and C. Rich. Formation, mineralization, and resorption of bone in vitamin D-deficient rats. J. Clin. Invest. 49:1122-1134, 1970.

4. Jowsey, J., et al. Quantitative microradiography studies of normal and osteoporotic bone. J. Bone Joint Surg. 47-A: 785-806, 1965.

5. Morey, E.R. Spaceflight and bone turnover: correlation with a new rat model of weightlessness Bio Science 29: 168-172, 1979.

6. Spengler, D.M., E.R. Morey, D.R. Carter, R.T. Turner, and D.J. Baylink. Effect of spaceflight on bone strength. The Physiologist 22: 575-576, 1979.

7. Wolff, J.: Das Gesetz des Transformation der knochen. Berlin, Hirschwald, 1892.

VALIDATION OF A NEW METHOD FOR STUDYING THE EFFECTS OF VIBRATION ON THE PRIMATE SPINE

P. QUANDIEU - C. NOGUES

Laboratoire Central de Biologie Aérospatiale
Section Biomécanique - 5bis, avenue de la Porte de Sèvres
75731 - PARIS AIR XV

In 3 years, 17 animals have been equipped with miniature accelerometers. Only 4 animals were retained for final results and interpretation: these were animals for which 9 validity test were positive.As long as all these conditions were met, results could be considered as significant.They can be summarized as follows:
when stimulated by vibration, the intervertebral disc behaves as a low-pass filter: this filter is linear, even when the amplitude of stimulation acceleration reaches relatively high levels (0.4 G).
Muscle activity causes a variation in the disc's transfer properties: muscle contraction improves its low-pass properties (lower transmission in the high-frequency range); muscle relaxation causes a translation of filter properties towards the high frequency range (transmission at 80 Hz improves with nembutal and curare).
Removal of the nucleus pulposus causes a shift in resonance to the high frequency range, the occurrence of non-linearity and a serious increase in transfer towards the high frequency range of the lumbar discs nearest the sacrum.

The type of acceleratory and vibratory aggression encountered in aeronautics, and during launch and reentry phases of manned spaceflights, is often exceptionally intense. This has led us to devise a way of studying the consequences of this type of aggression on the spinal column.

Our studies were carried out in the laboratory. We took a light-weight primate and implanted miniature accelerometers anteriorly on its vertebral bodies. These devices allowed us to study the mechanical behaviour of the lumbar spine when the animal, seated and subjected to medium-amplitude vibration stimuli, gave a muscle response under the effect of injected pharmacodynamic drugs.

The surgical technique involved consisted in screwing clips onto the anterior surface of the vertebral bodies. The miniature accelerometers were then inserted into these clips.

The equipment used to study the effects of vibration on the lumbar column is shown in the first slide. Among other items it includes a real-time two-channel analyzer able to calculate the transfer function of the intervertebral discs, as well as their correlation and coherence functions.

Any attempt to make a physiological interpretation of such an experiment is fraught with error unless it can be absolutely certain that the obtained signals are valid. The question : how valid are the structure's dynamic responses ? basically depends on how well the accelerometers have been fixed into position.

Checking that the transducers have been properly positioned is carried out in three stages : - radiology - assessment of the working behavior of the transducers - histology of the vertebrae.

X-ray examination. The purpose of this investigation is to check that the transducers are all on the same plane. When checking for a fault in parallelism between transducers, one evaluates the amount of error on the amplitude of the signal linked to the degree of transducer slant relative to gravity (absolute error) and to the neighbouring detectors (relative error). This error varies as the cosine of the angle by which the transducers are out of plane. In the example shown here, the absolute maximum error is 8° ,which is equivalent to a 1 % error in signal amplitude (cos 8° = 0.99).

This error is no greater than the noise from the magnetic recorder. It can therefore be considered acceptable.

Second test of validity: harmonic distorsion analysis. It is studied in a 0-100 Hz analysis band. Harmonics amplitudes are given in dB, and the 16 Hz main supporting signal amplitude of each spectrum is taken as a 0 dB reference value.

In an animal in which the transducers were left in situ for a very long time (approximately 45 days), we found when they were rejected that :
- harmonic distorsion analysis showed high non-linearity in the most securely positioned transducers. The lowest distorsion values were in signals from the least well anchored acceletometers.

These results are a little surprising, but not necessarily contradictory. Harmonic distorsion analysis is awkward to interpret, since it brings together under one roof distorsions in the excitation signal (vibrating table), the transfer characteristics of the structure being studied, and the quality of transducer anchorage. (The transducer is used in a frequency band in which it is perfectly linear).

Lastly, absolute certainty of proper transducer positioning comes from histology of the anatomy involved. This is the third test of validity.

A macroscopic view of a clip screwed into a vertebra shows that the depth of the screw is not greater than one half the thickness of the vertebral body.

Histology by Dr NOGUES C. has shown that implantation technique is improved by drilling the hole in the bone cortex immediately prior to transducer implantation.

Histology of samples taken from the vertebral bodies in the transducer implantation area shows two

different types of lesion. The first (without drilling) consists of microfractures of bone lamellae in regenerating fibroblast tissue. Regeneration is not yet complete and, in the zone of damaged tissue, conglomerates of fibrin and necrotic cells can still be seen. This lesion seems to have been caused by compression of spongy bone during implantation. The second (with drilling). The second type of lesion is more limited and involves only the immediate area of bone loss caused by transducer implantation ; necrosis is minimal, and there is no secondary infection. Even the vitality of the bone lamellae in contact with the transducer does not seem to have been endangered, and hematopoiesis is maintained in the implantation area.

Thus, the conclusion does seem to be that HISTOLOGY IS THE BEST WAY OF CHECKING THAT THE TRANSDUCERS HAVE BEEN PROPERLY POSITIONED.

Confidence in the validity of the obtained signals and in the processing system rests on :
- linearity - stationarity - ergodicity.

I would like to remind you, if I may, that as long as the condition of linearity is met, the disc operator is a convolution operator. If the conditions of stationarity and ergodicity are met, then the use of the Fourier transform over a finite period is justified.

Linearity studies were carried out in several tests.

a) Study of harmonic distorsion shows that the total level of distorsion is about 3 % for stimuli of the order of a few tenths G. But the investigation loses a lot of its usefulness because the excitation signal is already rich in harmonics itself and consequently, in the level of disc distorsion, it is impossible to distinguish disc-based non-linearities from non-linearities due to the excitation signal.

b) Theoretically, a system is linear if it complies with the principle of superposition.

In this relationship there are two features :
- homogeneity : if input is multiplied by a same constant factor, output is also multiplied by this same factor (effect proportional to cause).
- additivity : output resulting from various inputs is the sum of outputs resulting separately from the inputs (the effects of causes are additive).

Study of linearity under the heading of homogeneity only (effect proportional to cause) gives excellent results.

Accelerometer signals are stored on magnetic tape after digitalization. First- and second-order moments are then calculated.

Homogeneity was tested in the second-order moment. The first-order moment is theoretically equal to zero (the mean of a sinusoidal signal can be considered perfectly acceptable, since it is well within the order of precision of the equipment, normally between 1 and 2 %).

c) Coherence function: the transfer function, as calculated by the SD 360 analyzer, is a linearization of the biological model, since it is a coherent transfer function. This approximation can be measured through the study of the coherence function.

If the coherence function value is less than 1, three possibilities can be considered:
1) the system connecting the two signals (the intervertebral disc) is not linear ;
2) the system is linear but noise interferes with the signal ;
3) noise interferes with a non-linear signal.

Yet, the reverse does not hold: a coherence function equal to 1 does not ipso facto imply linearity.

Several types of stimulation (sinusoidal, sum of sine, sinusoidal sweep) give coherences of 1 on the stimulation frequency without the system being linear.

In our experiments, coherence is significantly decreased when transducers are incorrectly positioned. When properly fixed, the coherence function is greater than 0.99.

The two low coherence function values at either end of the band are related to stimulation of insufficient intensity, causing a decrease in signal/noise ratio.

Only those experiments were retained in which coherence was equal to 1.

d) Transformation of Gaussian signals."One of the essential features of GAUSSIAN signals is that they retain their GAUSSIAN character through any linear transformation, particularly through any linear filtering process".

Here again, the reverse does not hold: obtaining a GAUSSIAN signal when stimulating a system with a GAUSSIAN signal does not imply that the system is linear. In contrast, obtaining a non-GAUSSIAN signal is an absolute proof of non-linearity. We have been able to show the opposite by stimulating an animal with random vibration: the histogram of amplitude distribution was a GAUSSIAN distribution. Analysis of the signal from the vertebra furthest from the vibrating table (L3) shows good matching between the histograms.

The considerable agreement between results obtained in these various tests on stable animals shows good linearization of the disc filter, when the latter is stimulated by even fairly high levels of vibration (in our study, less than or equal to 0.5 G).

Now that the linearity problem can be considered settled, what are the statistical features of the signals obtained from the disc system ?

Applying the methodology, study of these statistical features means applying the protocol shown as a diagram on this transparency.

Besides studying linearity in signal recording, the time averages for a given physiological state can be compared, by repeating the experiment over the two following days, with those for a fixed-amplitude vibration stimulus (stationarity). Finally, the comparison between time averages and space averages tests the system's ergodicity.

Time averages were obtained from 50 or 100 effective acceleration values received from different vertebrae stimulated by different levels of sinusoidal vibration : 0.1 - 0.2 - 0.4 G at 18.7 Hz.

The study of time averages gave most satisfactory results.

This study also brought to light two facts that we might have expected.
- stationarity is all the more adversely affected as the stimulating level is higher and the output signal is further away from the input signal.

CHRONIC ACCELERATION AND BRAIN DENSITY

L. F. Hoffman and A. H. Smith

Department of Animal Physiology, University of California
Davis, California 95616 USA

The biological responses to gravitational changes are various. Some indicate a direct response to the altered load [7] -- e.g., bone and muscle, where a marked hypertrophy results upon increasing the load they must support. There are other responses commonly observed in chronically accelerated animals in which a similar load-response relationship is not apparent -- indicating a transduction of the original gravitational stimulus. Examples of these latter effects are decreases in growth rate, selective reduction of body fat, transient anorexia, impaired thermoregulation and an increased plasma protein concentration. Superficially, the transient anorexia, decreased growth rate and loss of body fat resemble the results of lesions in the lateral hypothalamus [4,8] -- and this has suggested that the hypothalamus may be involved in the transduction of the gravitational stimulus to produce these effects.

The brain is partly supported by the buoyant effect of the cerebrospinal fluid, so that in situ it has only a minor load -- e.g., in the 1500 gram human brain, a negative buoyancy of about 50 grams [6]. However, this load will be proportional to the ambient gravitational field -- so that at 2 G, it will approximate 100 grams. This load must be borne by brain tissue, e.g., the hypothalamus, potentially transducing the field stimulus and producing metabolic responses.

METHODS

Mature Polish rabbits (mixed sexes, and about 3 years of age) were exposed to chronic acceleration with a graded schedule that permitted physiological adaptation to a 2.5 G field. After various times at 2.5 G, animals were sacrificed and the brain and samples of the cerebrospinal fluid and heart ventricle were removed. Density of brain (D_{brain}) and myocardium (D_{myo}) were determined by comparing the weight of the tissue in air (W_{air}) and under water (W_{water}) -- the principle of Archimedes. Multiplying this specific gravity by the density of water at 20°C (0.9982) yields the final tissue density, corrected uniformly to 20°C:

$$\text{Density} = \frac{W_{air}}{W_{air} - W_{water}} \times 0.9982$$

Cerebrospinal fluid density (D_{csf}) was determined with a petroleum ether-carbon tetrachloride density gradient column, calibrated with standard copper sulfate solutions (checked with a sensitive hydrometer), and corrected to 20°C. From brain mass (M_{brain}) and density it is possible to calculate the mass-specific brain load (negative buoyancy) as grams weight per 100 grams brain mass:

$$\frac{M_{brain} - [D_{csf} \times (M_{brain}/D_{brain})]}{M_{brain}} \times G \times 100$$

RESULTS/DISCUSSION

The effect of chronic acceleration was not uniform among the various tissues studied. Although body mass was reduced by the treatment, as expected, no change was apparent in brain mass or in the density of cerebrospinal fluid (Table 1). Acceleration-induced changes were encountered in tissue density, the myocardium exhibiting a transient increase followed by an exponential decrease toward a limit and the brain showing an arithmetic increase in density with continued exposure to 2.5 G (Table 2). The values for brain and cerebrospinal fluid densities in control animals -- 0 time at 2.5 G -- are comparable to, or within the range of values reported by others for rabbits [1,3,5].

Acceleration-induced changes in brain load are observed considering the load in a 1 G environment (Table 3), and these were found to be related to treatment time at 2.5 G (t):

$$\text{Mass-Specific Brain Load(at 1 G)} = 3.41 + 0.011t$$
$$[n = 5,\ r = 0.981,\ p < 0.01]$$

Of course, the actual brain load for the centrifuging animals is 2.5-times that of the 1 G load (Table 3), and increases toward a limit with exposure time:

$$\text{Mass-Specific Brain Load(at 2.5 G)} = 9.45 - 0.061e^{-0.13t}$$
$$[n = 5,\ r = 0.981,\ p < 0.01]$$

These data suggest, then, that specific brain load is not a regulated phenomenon, and no physiological processes occur to attenuate the increased load imposed by the hyperdynamic environment.

Table 1. Mean body size, brain size, and cerebrospinal fluid density (D_{csf}) of chronically accelerated and control rabbits (± standard deviation).

	(n)	Centrifuged	(n)	Control	(n)	All Observations
Body Mass (kg)	(16)	1.61 ±0.11	(7)	1.97 ±0.10		-----
Brain Mass (g)	(16)	8.30 ±0.34	(7)	8.33 ±0.32	(23)	8.31 ±0.33
D_{csf}, 20°C	(9)	1.0039±0.0006	(4)	1.0039±0.0011	(13)	1.0039±0.0007

Table 2. Mean brain and myocardial tissue densities of chronically accelerated and control rabbits (± standard deviation).

Time at 2.5 G (days)	(n)	TISSUE DENSITY Brain	Myocardium
0	(7)	1.0391±0.0014	1.0480±0.0042
12.8±1.7	(4)	1.0412±0.0028	1.0668±0.0152
19.8±1.5	(6)	1.0415±0.0014	1.0515±0.0034
26.3±1.7	(4)	1.0426±0.0017	1.0479±0.0022
35.0±4.4	(3)	1.0431±0.0024	1.0467±0.0029

Table 3. Mass-specific brain load, calculated from mean density at the exposure periods indicated. The value for D_{csf} is the mean for all observations.

Time at 2.5 G (days)	D_{brain}	D_{csf}	Specific Load 1.0 G	2.5 G
0	1.0391	1.0039	3.388	-----
12.75	1.0412	1.0039	3.582	8.955
19.83	1.0415	1.0039	3.610	9.025
26.25	1.0426	1.0039	3.712	9.280
35.00	1.0431	1.0039	3.758	9.395

The inter-species influence of body size on the physical properties of the brain and cerebrospinal fluid also is of interest. Some available data [1,2,9] indicates the following relationships between body mass (BM, kg), brain mass (M_b, gms) and densities at 20°C of cerebrospinal fluid (D_{csf}) and brain (D_b):

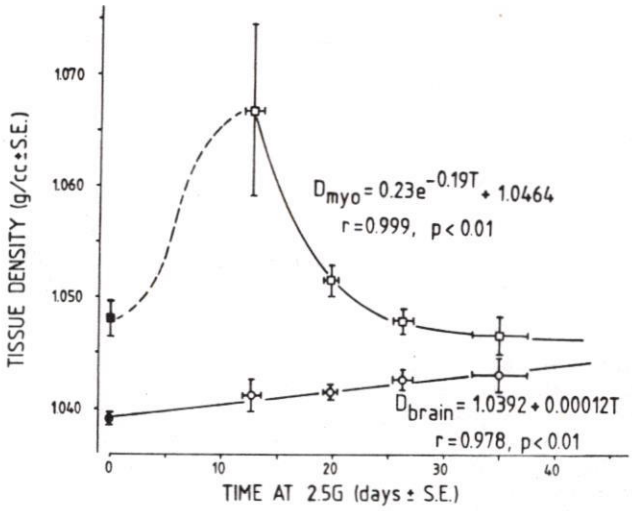
$$M_b = 6.71\ BM^{0.68}$$
$$[n = 10,\ r = 0.970,\ p < 0.001]$$
$$D_b = 1.0483 - 0.0027 \log BM$$
$$[n = 8,\ r = -0.636,\ ns]$$
$$D_{csf} = 1.00353 + 0.00047 \log BM$$
$$[n = 11,\ r = 0.639,\ p < 0.04]$$

Not enough of these data are on the same species to permit a direct estimate of brain load, but from the above equations it appears that the mass specific brain load (L_m, gms load per 100 gm mass) decreases with body size:

$$L_m = 4.25\ BM^{-0.032}$$

However, transduction of the load stimulus would more likely be a function of the surface specific brain load (L_s, gms load per 100 cm^2 brain surface, calculated considering the brain as a sphere: $S = 4.8\ V^{2/3}$). This would be proportional to the pressures generated by the brain load:

$$L_s = 1.76\ BM^{0.18}$$



This equation indicates that the stimulus potential per unit of brain load increases with body size -- even though brain density decreases and cerebrospinal fluid density increases. This is consistent with the general observed greater effect of acceleration (reducing body size and fat content) with increasing body size.

ACKNOWLEDGEMENT

This research was supported in part by a NASA Grant: NSG 7493.

REFERENCES

1. Altman, P. L. and D. S. Dittmer, 1974. Biology Data Book. 2nd ed. Vol. III, pp. 1976-80. FASEB (Bethesda).
2. Butler, T. M. and G. L. Wiley, 1971. Baseline values for adult baboon cerebrospinal fluid. Lab. An. Sci. 21(1):123-24.
3. Chiao, T. and L. Chun-Yu, 1939. The density of animal tissues. Chinese J. Physiol. 14(1): 39-50.
4. Keesey, R. E., P. C. Boyle, J. W. Kemnitz, and J. S. Mitchell, 1976. The role of the lateral hypothalamus in determining the body weight set point. pp. 243-56 in: Hunger: Basic Mechanisms and Clinical Implications. D. Novin, W. Wyrwicka, and G. A. Bray, Eds. Raven Press (New York).
5. Leith, J. T. and J. F. Gaugl, 1972. Changes in specific gravity of rabbit brain after high doses of x-irradiation. Int. J. Radiat. Biol. 21:573-82.
6. Ruch, T. C. and H. D. Patton, 1965. Physiology and Biophysics. 19th Ed. Saunders (Philadelphia) p. 938.
7. Smith, A. H., 1978. Gravitational Physiology. The Physiology Teacher 7(1):4-13.
8. Smith, A. H. and M. J. Katovich, 1979. Gravitational influences upon the maintenance requirements of rabbits. COSPAR Life Sci. Space Res. XV:257-61.
9. Webb, A. I. and B. M. Q. Weaver, 1979. The density of equine tissue at 37°C. Res. Vet. Sci. 26:71-75.

GRAVITY ONLY DEPENDENT RECEPTOR FIELD OF THE VESTIBULAR SENSORS: ITS SIGNIFICANCE IN ORBITAL FLIGHT

Gualtierotti,T.

ISTITUTO DI FISIOLOGIA UMANA II UNIVERSITA' MILANO,MILAN (Italy)

SUMMARY

In the bull frog the gravity dependent receptive field of tonic and phasic-tonic vestibular receptors covers nearly the entire 360° solid angle. Consequently any given position of the head will modify the adapted firing rate of the entire family of statoreceptors,in an orderly pattern.
As a result, stationary responses to gravity effectively modulate the evoked activity of the vestibular receptors due to head movement. In "0" g this organized gravity dependent pattern disappears altering the amplitude and mode of the vestibular responses to head movements. The problem is further complicated by the random "wandering" of the cell mean frequency which is orders of magnitude larger in "0" g than on earth.

In the bull frog tonic and phasic-tonic vestibular statoreceptors (fig.1) show gravity dependent only receptive fields that cover nearly the full 360° solid angle (fig.2). Pure phasic receptors do not show any response to gravity vectors (1-2).

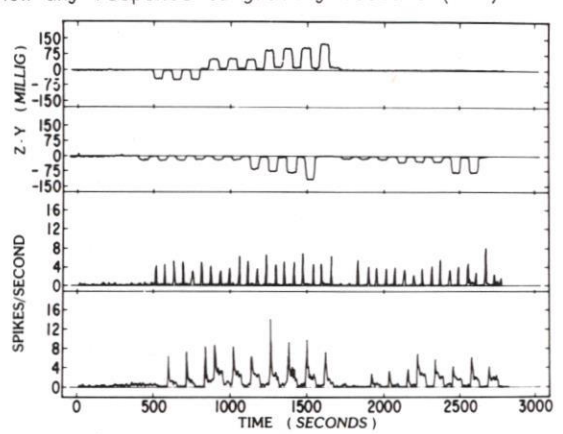


Fig.1 Response to tilt of a purely phasic vestibular receptor (3rd record from top) and a phasic-tonic statoreceptor (last record).

A phasic response only is recorded from the phasic receptor whereas the phasic-tonic receptor shows both a tonic and a pronounced phasic phase. From top:1°and 2°records Z-Y acceleration; abscissa:time in seconds.

In this paper the response to gravity of tonic and phasic-tonic receptors only will be considered. Their receptor field consists of two halves,one positive(increase of firing rate)and one negative(decrease of firing rate)divided by 2 opposite limited solid angles of few degrees in which no response to the rotation of the gravity vector is recorded(fig. 2:N.P.=Null Point).Normally the Null Points are situated at 90°approx in respect of the position in which the peak responses are recorded.It is considered that at peak response the polarization vector of the cell is coincident with the gravity vertical. Within the field non linear symmetric distributions with a central peak or asymmetric ones with on off center peak are found.Very often the negative half of the receptive field is only partially developed as the rate of firing is rapidly reduced to 0(fig.2)

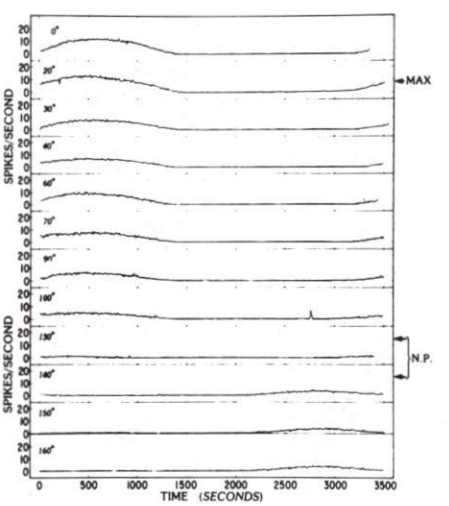


Fig.2 Receptive field of a phasic-tonic vestibular statoreceptor during sixteen 360°vertical rotation at 10°horizontal intervals one from the other.Note:in the bench position (origin of the records on left)an adapted frequency of discharge is maintained. During the vertical rotation the field is limited to the positive phase,as at the onset of the negative field the firing is quickly inhibited by the head down rotation.Maximal response (arrowed

in the records) is recorded at 20°horizontal. Null Point (N.P.)starts at 130°at right angle with the plane of maximum response.On the ordinates:spike/second,on the abscissa:time of vertical rotation in sec:as the angular speed was constant,at 0.1°/sec,by dividing by ten the values on the abscissa the degrees of vertical rotation are obtained;e.g.2500 sec.=250°.Numbers on left on each record=degrees of horizontal rotation.

As the ± receptive field of each receptor is practically spheric, a given position of the head in the earth gravitation field will modify the activity of all statoreceptors,(except the few at Null Point in that particular situation)in an orderly pattern:positively or negatively,according to the angle between the gravity vertical and the polarization vector of each cell.The resulting activity will be"adapted" as a function of the gravity input(3):$\bar{m}$ sin a. $\bar{m}$=average firing rate;a= angle between the gravity vertical and the polarization vector of the cell.

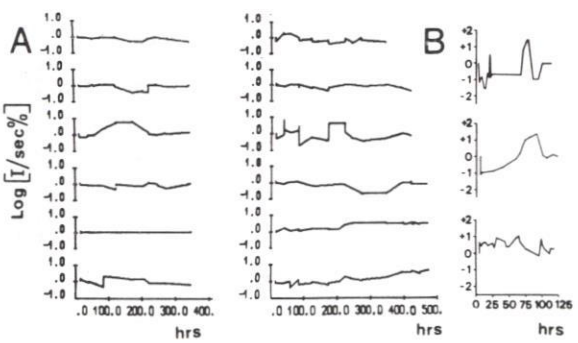


Fig.3 Mean frequency of the adapted firing rate of A) 12 vestibular units on ground and B) of 3 units in orbit.On the ordinate:log of the mean frequency values exceeding the starting level;(1=x 10;2=x 100;-1=1/10;-2=1/100). On the abscissa time of recording in hours: as shown significant "wandering" of the mean firing rate appears at different time and duration in all units.In the units in orbit however it is of orders of magnitude larger than in the ones on the ground.

On this basic adapted activity any further stimulus resulting from head movement will be superimposed. Consequently stationary responses to gravity effectively modulate the evoked activity of the vestibular receptors following head movements as the gravity vectors modify the adapted rate of firing corresponding to any given starting position of the head. For instance the unit represented in fig.2 will respond to a negative stimulus by decreasing the rate of firing only if the starting position of the head corresponds to the positive half of the field: the negative response will be maximal at the positive peak and absent when the adapted rate of firing is already "0".
In "0" g the gravity dependent adaptation of the activity of the tonic and tonic-phasic vestibular receptors disappears while phasic ones will remain unchanged and therefore the amplitude and mode of the responses to head accelerations change significantly and sistematically. The problem is further complicated by the random wandering of firing at rest, which is much larger (3-4 x)than on earth (4) (fig.3). This last factor is bound to confuse the central analysis of the peripheral activity as it is not possible to distinguish between changes of the firing rate of a given cell due to wandering and the ones due to the stationary starting position of the head: this in a gravitational field is indicated by the frequency pattern of the entire family of peripheral receptors. In effect "wandering" happens independently for each receptor within the end organ (5).

REFERENCES

1) Gualtierotti,T.,Bracchi,F. & Morabito,A.1980 The gravity dependent receptor field of single vestibular statoreceptors in a physiologically stable environmentally controlled bull frog preparation.Abstr. XXVIII IUPS 1647: 450

2) Gualtierotti,T.,1969; Unidirectional response of statoreceptors to vibration. Proc. 8th International Symp.on Space Technology and Science, Tokyo pp.1193-1200.

3) Lowenstein,O.,The origin and functional significance of the resting activity and peripheral adaptation in the vestibular system.From Gualtierotti, T.,1981 The vestibular system.Function and Morphology, Springer Verlag,N.Y. pp.317-326.

4) Bracchi,F.,Gualtierotti,T.,Morabito,A.& Rocca,E 1975 Multiday recording from the primary neurons of the statoreceptors of the labyrinth of the bull frog. Acta Otolaryngol. S.334: 1-27.

5) Bracchi,F.,Morabito,A.,Gualtierotti,T.,Variability of the spontaneous firing rate of vestibular receptors in a stable controlled bull frog preparation. From Gualtierotti,T.,1981 The vestibular system.Function and Morphology, Springer Verlag, N.Y.pp.329-336.

RELATION BETWEEN PHYSIOLOGICAL EFFECTS OF GRAVITATIONAL FORCES AND THAT OF MAGNETIC FORCES—II

H. Saiki, M. Sudoh, M. Nakaya and M. Abe

Space Medicine Laboratory
The Jikei Univ. Tokyo, Japan, 105

INTRODUCTION

In terrestrial and outer space environments, there are various intensities of geographical and artificial electric magnetic fields. On the biological effects, there are excellent reviews by Busby,[1] Barnothy,[2] Presman[3] and Wladimirsky,[4] as were quoted in 1978.

We had reported about experimental results in comparison of responses of such physiological functions as wound healing function, swimming capacity of mice during and after low magnetic field (LM) exposure, under simulated hypogravic conditions, to those of geomagnetic field exposure, as the 1st report under the same title at 28th IUPS meeting last year.[5] And we took our attention to the influences of unusual magnetic fields. At the case of normodynamic and hypodynamic life, as in our previously reported paper, it was concern to the low magnetic fields. But in this report, high magnetic fields of some level were used as the unusual magnetic conditions. Such reports, combined with our scheduled experiments on the hypergravic life, expected to compose a part of our entitled studies.

EXPERIMENTAL METHODS

We are going to report that the effects of various intensities of magnetic field such as LM, geomagnetic field (GM), and high magnetic field (HM) exposure on the metabolic and cardiovascular functions of albino rats under same simulated hypogravic and normogravic conditions. Hypokinetic albino rats of Wister King strain, induced by orthostatic suspension technique, were exposed for 3 weeks to geomagnetic field (Ca 640mG), low magnetic field (Ca 4mG) by active shielding and high magnetic field (Ca 200G) by ferrite magnet, respectively. The process of adaptation to hypokinesia for 3 weeks and process of recovery after termination from hypokinesia to normal ambulatory life were observed for 7 weeks. As test animals, we used female rats of 10 weeks age by previously reported reason.[6]

Through all the experiment, daily urine volume, daily urinary excretions of Na^+, K^+, and Ca^{2+}, blood pressure, pulse rate, rectal temparature, and resting metabolic rate were measured. And data of 3 different intensity magnetic field exposure groups were compared with each other. In these experiments, we were enforced to use a kind of cover for the sustainment of ferrite magnet. In the apparatus for ferrite magnet exposure, two ferrite magnets of 12 cm by 20 cm in size fixed on the aluminun cover at 9 cm interval. The cover was set over the suspended animal in parallel to the body axes. Such magnets produce about 200G at the center between the two magnets, where the center of the animal body were fixed. For GM and LM group, the aluminum cover is of the same size and contains the Bakelite plates in stead of the magnet. And we found that the resting metabolic rate of such covered animals were significantly lower than without. So that, we performed another experiments to clarify this point.

RESULTS

1. Body weight and blood pressure: The fluctuation of these parameters of the 3 groups were expressed in percent of values observed during the pre-hypodynamic exposure period. Blood pressure was etimated at the tail artery by electro-sphygmo manometer (PE-300 Narco Bio-Systems Inc.) Body weight as well as blood pressure showed no clear differences between each of the GM, LM and HM groups. But, the body weight curves show the transient decrease and return to the pre-hypodynamic exposure values. During the recovery period, body weight increased very sharp. The HM group shows the lowest curve during hypodynamic exposure, and during recovery shows the steepest increase. This suggests that the influences of hypodynamics was more pronounced than other groups. As for the blood pressure curve, it increases during the hypodynamic exposure to a higher level than pre-exposure control values and during the recovery came down to the original level within 3 weeks.

2. Urinary excretion of K^+ and Ca^{2+}: On the urinary excretion rate of potassium expressed as the relative fluctuation of weekly mean value, K^+ decreases during the 1st week, then increases over the pre-hypodynamic period value and reaches the summit of 129% in the case of the GM group, and during recovery it increases, following the sharp decrease of the 1st week, and increased level for 4 week return to the pre-exposure level at 5 weeks recovery period. In the case of HM, the increase was very fast from the beginning of exposure and through the slight transient decrease reached the comparatively high level static plateau. During the recovery period, they return to pre-exposure level by the fourth week. As for the LM, during the exposure period, K^+ excretion rate have the tendency of decreasing and do not show a tendency to increase. They return to the pre-exposure level within 4 weeks. As for the LM, the release of K^+ from muscle is not to be expected. A rapid attainment of a plateau seems to be a characteristic of HM. In the urinary excretion of calcium expressed as the relative fluctuation of weekly mean value, a similar tendency was observed as in the case of K^+.

But the increase in the 1st week was not observed in the case of HM group, the progressive decrease of Ca^{2+} in the bone tissue. The time for recovery to the pre-exposure level is most rapid in the case of the LM group, and is slowest in the case of the GM group. Considering such characteristic of urinary excretion of K^+ and Ca^{2+}, the GM and HM group have more tendency to lose K^+ and Ca^{2+} from the body than LM group.

3. Resting metabolic rate and urine volume: Fig. 1 shows the fluctuation of daily urine volume and the resting metabolic rate measured by indirect calorimetry at the same time of every second or third day. The values of the pre-hypodynamic exposure period were expressed as 100%. Resting metabolic rate decreased during hypokinetic exposure and then has tendency to increase and approach to the pre-exposure value at the end of 3 weeks of the exposure. During the recovery phase, they show a transient high value, and come back to the original level by the seventh week. With respect to the values during exposure to hypodynamics and various magnetic forces, the GM group shows the smallest decrease, the HM group shows more pronounced decrease which is significantly different in one point. Between the GM group and the LM group, no differences were observed statistically. With respect to the changes of metabolism, we had reported that orthostatic suspension produce the hypermetabolism in stead of hypometabolism. In this experiment, we found the opposite. However, in this experiment we used, for the first time, special cover. About the influences of the covering, I will report afterward. With respect to the urine volume, orthostatic condition does not produce polyuria during the 1st week of exposure. But, there is a tendency of decrease without the HM group, and during the 3rd week of exposure, a clear tendency of increase or recovery was observed in all groups. We could find a tendency of polyuria, in the case of some kind of anti-orthostasis, and this covered orthostasis. The increase of urine volume during 2, 3 weeks is more rapidly than in the GM group. LM group showed no such phenomina during the 2 weeks. Any way, in the case of hypodynamics by suspension technique, the 1st week must be a stress phase. After that, this specific effects of hypodynamics became manifest. As the next step, we investigated the influences of cover on the metabolic rate.

4. Comparison of covered and non-covered group: The metabolic rate of covered and non-covered rats during orthostatic suspension for 3 weeks, showed clear differences between the two groups were statistically significant in some parts of the curve. We can see clear decreasing by covering. For the next step, the comparison fo seasonal changes of metabolic rate, between covered and non-covered groups were performed. In winter time, the non-covered group shows a tendency to increase, but the covered group has not such tendency. However in summer time, even the non-covered group, still shows no increase but covered group shows a clear decrease. Some values showed a clearly statistically significant difference between the two groups. Our experiment were done in winter time. If we had performed it in summer time, the covered condition maybe would have decreased the metabolic rate during the hypokinetic conditions markedly. So we can assume that the covered condition does not oppose the influences of hypokinesis, but acts in the same direction.

SUMMARY

The findings on different parameters can be summarized as follows:

1. HM has a tendency to promote the decreasing effects of hypodynamic exposure in such parameters, as resting metabolic rate and urine volume.

2. LM has a tendency to attenuate the effect of the hypodynamic exposure in such parameters as urine volume, K^+, and Ca^{2+} excretion rate. These findings are supported by the swimming exercise capacity of the mice, that had been reported by us.[5] During the recovery period, return to the control values is attained earlier with LM than with GM or HM.

3. For the simulation of hypogravics, there is a possibility that effect is promoted by covering, especially for metabolism.

To clarify the relationships of gravitational effects and magnetic forces, we are continuing to study wider ranges of such factors, using different kinds of biomedical subjects.

REFERENCES

1) Busby, D.E. 1968. Space biomagnetics. Space Life Science Vol. 23-63.

2) Barnothy, M.F. 1964, 1969. Biological effects of magnetic field Vol.1,2. Plenum Press, New York and London.

3) Presman, A.S. 1970. Electromagnetic fields and life, translated by F.L. Sinclair, Plenum Press, New York.

4) Schmidtke, G. 1978. Personal communication.

5) Saiki, H., M. Saiki, M. Nakaya, M. Sudoh, M. Abe and Y. Taketomi. 1980. Relationship between physiological effects of gravitational forces. The Physiologist Vol.23 No.6 s149-s150.

6) Morris, D.J., J.S. Berek and R.P. Davis. 1973. The physiological response to a aldosterone in adrenalectomized and intact rats and its sex dependence. Endorinology Vol.92 989-993

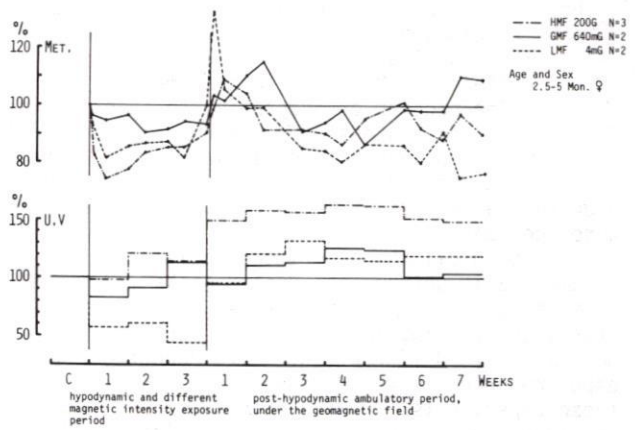


Fig. 1. Fluctuation of metabolic rate and urine volume. (The values of pre-hypodynamic exposure period were expressed as 100%)

CORE TEMPERATURE AND BRAINSTEM AUDITORY EVOKED POTENTIALS AS COMPLIMENTARY NONINVASIVE MEASURES OF CENTRAL NEURAL FUNCTION DURING EXPOSURE TO HYPERGRAVIC FIELDS

T. A. Jones and J. M. Horowitz

Department of Animal Physiology
University of California, Davis
Davis, California 95616

Introduction.

Several lines of evidence clearly show that central thermoregulatory mechanisms are impaired in rats during exposure to orthodynamic hypergravic fields of +2Gz to +3Gz (2,4). The intention of the present study was to determine if the function of another system, the auditory system, is also impaired during exposure to the same gravitational field profiles. Brainstem auditory-evoked potentials (BAEPs) were used to obtain an objective, noninvasive measurement of auditory function in free moving unanesthetized animals.

The technique of recording BAEPs as originally described by Jewett and coworkers in 1970 (3) is now commonly employed by physicians to assess brainstem function. BAEPs (like the electrocardiogram) are voltages commonly measured at considerable distances from the sources. In the case of BAEPs there is a marked attenuation of the potential field conducted from the brainstem through the brain parenchyma, skull and skin to scalp recording electrodes. BAEPs are one to two orders of magnitude smaller than spontaneous electroencephalographic (EEG) activity yet can be isolated using computerized averaging techniques. The various components (I-V) of the BAEP waveform (figure 1) have been shown to depend on particular portions of the auditory relay system between the ponto-medulary junction and the posterior thalamus (1,3).

One difficulty associated with the use of BAEPs in unrestrained animals is the control of stimulus parameters. Movement of animals relative to a fixed sound source can produce substantial variation in the latencies of BAEP components. Both stimulus intensity and the conduction time from the source to the ear may vary. These difficulties were avoided in the present study by developing a new means of presenting an acoustic stimulus via bone conduction. Following this development, studies were undertaken which examined the effects of +3Gz fields on BAEPs in rats.

Methods.

One cat and three rats were used in the present study. All surgeries were performed under pentobarbital anesthesia. Vertex, Mastoid and frontal sinus screws were placed for recording EEG activity. Calibrated thermistors were placed in the midbrain or thalamus of all animals and used to monitor brain temperature. Bone-conducted stimuli were produced by the vibration of a polycrystaline ceramic PZT (lead-zirconate-titanate). Coupling of the vibrations to the skull was accomplished by embedding crystals within an acrylic platform originally poured as a liquid over stainless steel skull screws. Animals were allowed to recover for at least seven days before testing.

During experiments EEG signals were amplified, led to an FM magnetic tape recorder and later a commercial digital averager was used to resolve BAEPs. Responses to 1024 clicks or to 1024 tone pips (approximately 60 dBHL) were averaged for all records. The rats were placed on the centrifuge in a thermally insulated, sound attenuated chamber for recordings. Ambient room temperatures were between 27 and 32°C. Recordings of BAEPs and brain temperature were made before, during and after +3Gz acceleration. Ambient noise was maintained at a constant level under all conditions.

Results.

Representative crystal-evoked brainstem potentials from the cat and rat at +1Gz are shown in figure 1. The BAEPs appear identical to potentials evoked by air-coupled stimuli. Wave I (figure 1, top) does not actually originate in the brainstem but rather represents the compound action potential of the cochlear nerve in response to a click or tone pip. The remainder of the waveform (waves II- V) is thought to be derived from the sequential activation of auditory structures located at progressively more rostral locations in the brainstem. The generators for individual components appear to be similar across species although later waves may be numbered differently in human studies. For BAEP components as numbered in cats, the source neurons are believed to arise within particular structures as follows: wave II: cochlear nucleus, III: superior olivary complex, IV: nucleus of the lateral lemniscus, and V: inferior coliculus (1).

A remarkable reproduciblility of BAEP components was acheived with the precisely controled crystal stimulus despite normal movement and behavior of the animals as shown by the superposition of traces in figure 1.

Table 1: Effects of hypergravic fields on BAEPs. Latency is given as the difference in time (in msec) between peak V and peak II (a measure of central conduction time) and the standard deviation (n=3, $x \pm sd$). Total exposure for each animal was 1.5 hours at +3Gz. Mean brain temperatures are also given (°C). No significant differences were found for any measure for +3Gz.

Condition	Latency	Brain Temperature
+1Gz (before)	1.3 ± 0.37	38.5 ± 0.9
+3Gz (0 to .5hr)	1.3 ± 0.38	38.3 ± 1.2
+3Gz (1 to 1.5hr)	1.3 ± 0.38	37.6 ± 1.3
+1Gz (after)	1.3 ± 0.44	37.9 ± 0.9

Table 1 summarizes the results for 3 rats before, during (first and last 30 min.) and after the 1.5hr exposure at +3Gz. rats. No significant differences in amplitudes or latencies were found between BAEPs at +1Gz and at +3Gz. A Large drop in temperature was avoided in these animals by maintaining ambient temperatures near 32°C. These data represent the first recording of BAEPs in animals under hypergravic conditions.

Discussion.

The present results, albeit preliminary, show no evidence of auditory impairment during +3Gz acceleration despite the marked alteration of thermoregulatory function in these animals (2,4). The measurements on the auditory system were performed under the same conditions as were measurements on the thermoregulatory system. That is, both sets of measurements on unrestrained, conscious rats were made using the same +3Gz exposure. The results demonstrate that measurements of temperature and BAEPs, when considered together, can provide complementary information since the measures reflect activity in distinct anatomical as well as functional systems. The feasibility of monitoring brainstem function noninvasively in conscious animals using BAEPs during acceleration is demonstrated in this study. The effects of higher intensity as well as 0.0g gravitational fields on BAEPs in both man and animals remain to be determined in future studies.

We thank Dr. A. H. Smith for making available the centrifuge facilities at the Chronic Acceleration Laboratory at UCD. This work was supported by NASA grant NSG-2234 and NASA 5-33146.

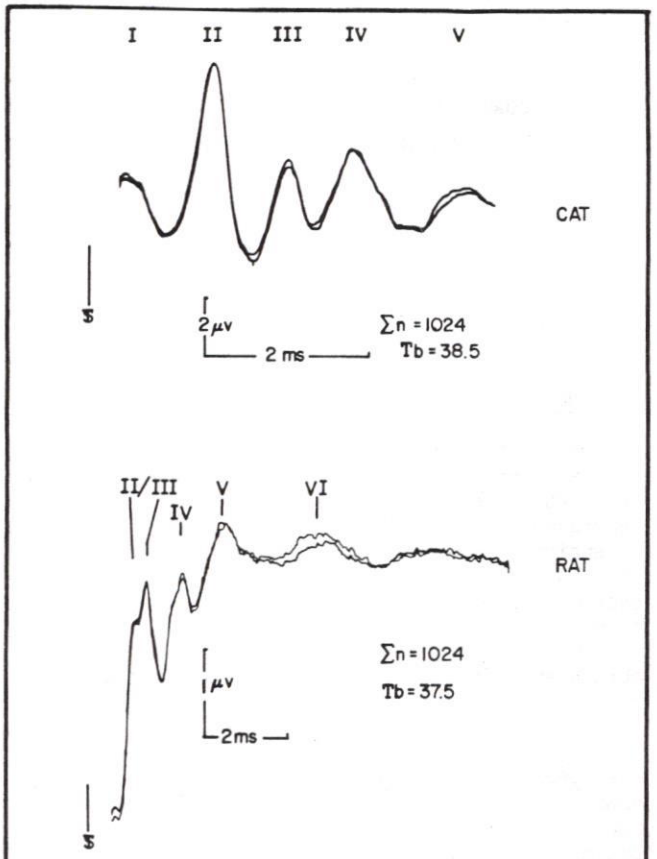


Figure 1: Examples of BAEPs recorded at +1Gz in the cat (upper traces) and rat (lower traces) in response to crystal-evoked, bone-conducted auditory stimuli. These and all animals of the present study were free-moving and unanesthetized. Two traces, each the average of 1024 responses (ΣN) are superimposed to illustrate the reproducibility of the response. μv= microvolts, ms = milliseconds, $ = stimulus onset. Wave I is partially obscured by the stimulus electrical artifact which is deleted. Traces are direct photocopies of original data. Positive voltages are plotted upward.

REFERENCES

1. Buchwald, J.S. and Haung, C.M., Far field acoustic response: origins in the cat. Science 189:382-384, 1975.
2. Giacchino, J., B.A. Horwitz, and J.M. Horowitz. Thermoregulation in unrestrained rats during and after exposure to 1.5-4G. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 46(6):1049- 053, 1979.
3. Jewett, D.L., M.N. Ramono., and J.S. Williston. Human auditory evoked potentials. Possible brainstem components detected on the scalp. Science 167:1516-1518, 1970.
4. Schertel, E.R., J.M. Horowitz, and B.A. Horwitz. Effects of gravitational profiles on the rat's thermoregulatory responses to cold. J. Appl. Physiol.: Respir. Environ. Exer. Physiol. 49:663-668., 1980.

RESPONSE OF CULTURED CELLS TO HYPER- AND HYPOGRAVITY

A. Tschopp[1], W. Briegleb[2] and A. Cogoli[1]

[1] Laboratorium für Biochemie, ETH-Zentrum, CH-8092 Zürich
[2] Institut für Flugmedizin, DFVLR, D-5000 Köln 90

INTRODUCTION

Simulations of hypo- and hypergravity conditions are useful tools for the planning and designing of biological experiments in space laboratories. Although true 0xg conditions cannot be achieved on earth, low-g can be simulated in the fast rotating clinostat(1). Previous experiments performed in our laboratories have shown that hypergravity (2-10xg) enhances, whereas hypogravity (0.2xg) depresses stimulation of lymphocytes by mitogens in vitro (2,3). These findings raised our interest in the behavior of other cell lines under gravitational stress.

In this paper we describe preliminary results obtained with four cell lines at high-g and with lymphocytes monitored in the fast rotating clinostat.

METHODS

Experiments were performed in a walk on breeding room at 37°C: High-g levels, in the range between 3 and 10xg were generated in a MSE table top centrifuge. Low-g conditions were simulated in a clinostat microscope (horizontal microscope, turnable around its optical axis, speed 100 rpm). 1xg controls were kept in a horizontal microscope. The microscopes were connected with a time lapse video recorder.

In the centrifuge cells were cultured either in flasks (25cm^2, Falcon) or in culture plates (24 wells, Nunc). In the clinostat cells were kept in a microchamber consisting of a slide and a cover slide sealed with beeswax, paraffin and vaseline. Lymphocytes were exposed to mitogenic concentrations of concanavalin A (2,3).

RESULTS

High-g simulations

Early experiments, especially when performed in cell culture flasks, showed an unequal distribution of cells on the surface. In fact, an inaccurate alignement of the flasks versus the centrifugal forces shifted the cells towards the corners of the flask. Therefore, the cells were allowed to adhere for 12 hrs before starting the centrifugation. This period of time permits also to overcome the lag phase of the cells.
A comparison of the growth rate of different cell lines at 10xg is shown on fig. 1. Growth was measured by incorporation of tritiated thymidine into DNA. This parameter correlates fairly well with the real cell number.

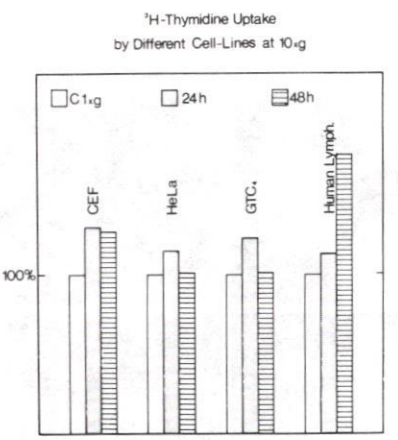


Fig. 1: ^{3}H-thymidine uptake by different cell lines, measured at 10xg at 24 and 48 hrs, compared with the respective 1xg control taken as 100%.
CEF : Chicken Embryo Fibroblasts
GTC_4: Galliera Tumor Cells Clone 4

Chicken embryo fibroblasts, HeLa cells, GTC4 cells cloned from sarcoma Galliera (a rat tumor) and human lymphocytes show a markedly increase of growth rate after 24 hrs of incubation at 10xg. Growth rate slows down at 10xg in HeLa cells and GTC4 cells after 48 hrs since the monolayer became confluent.

Preliminary results at high-g indicate that there is no correlation between g-level and cell growth, i.e. the extent of the high-g effect remains essentially the same at 3, 20 and 40xg.

Low-g simulations

In earlier papers we reported the behavior of human lymphocytes under simulated microgravity conditions in the fast rotating clinostat (2,3). We will investigate the activation of lymphocytes by mitogens in-vitro in a real microgravity environment on the next spacelab flights. Here we report preliminary observations on cell motility and aggregation monitored at the fast rotating microscope. Motility and aggregation are important factors affecting cell-cell interactions. It is known that intracellular interactions are regulating the activation of lymhocytes.

Although our observations are limited to only one preparation, it can be clearly seen from Fig. 2 that lxg lymphocytes tend to form compact aggregates whereas at low-g cells are looser. After approximately 13.5 hrs the tendency to form compact aggregates in the controls and looser cells in the low-g samples is more pronounced than at the beginning of the observation. In addition, aggregates at low-g are characterized by a higher number of in- and out passing cells.

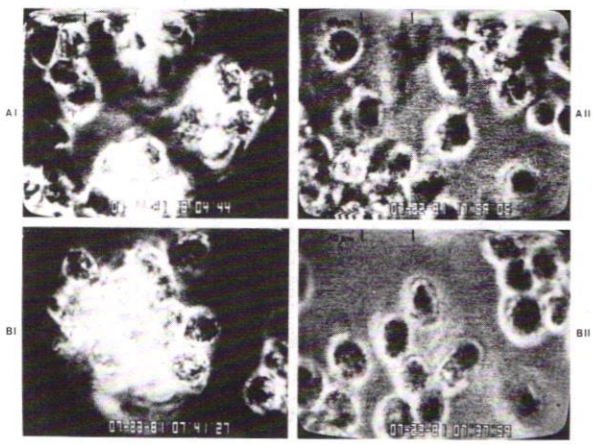


Fig. 2 shows the aggregation patters of lymphocytes exposed first for 47 hrs to the mitogen concanavalin A at lxg in Falcon cell culture flasks and then transferred into microchambers fixed either in the horizontal microscope (lxg condition) or the rotating microscope (low-g condition). Pictures AI and AII were taken after 2 hrs in the microscope, pictures BI and BII after 13.5 hrs in the microscope. AI and BI refer to the lxg culture, AII and BII to the low gravity sample.

CONCLUSIONS

Our experiments clearly show that gravity has an influence on cell growth, indipendently from the cell line. High-g increase the growth rate, whereas low-g have an inhibitory effect.

These findings may lead to interesting observations for basic research and to important applications in biotechnology (4).

ACKNOWLEDGMENT

This work was supported by the Swiss National Research Foundation, Bern, Grant No. 3.499-0.79. The skilful technical assistance of Pia Bislin is gratefully acknowledged.

REFERENCES

1. Briegleb, W., A. Schatz, and J. Neubert. Das Pendant zum Zentrifugenmikroskop: Klinostatenmikroskop. Umschau Wiss. u. Techn. (1976) 76:621-623.

2. Cogoli, A., M. Valluchi-Morf, H.R. Böhringer, M.R. Vanni, and M. Müller. Effect of Gravity on Lymphocyte Proliferation. In: COSPAR Life Sciences and Space Research XVII (1979) Pergamon Press Oxford and New York.

3. Cogoli, A., M. Valluchi-Morf, M. Müller, and W. Briegleb. Effect on hypogravity on human lymphocyte activation. Aviat. Space Environ. Med. (1980) 51(1):29-34

4. Cogoli, A., and A. Tschopp. Biotechnology in Space Laboratories. Advances in Biochemical Engineering. In press.

TEMPERATURE AND BEHAVIORAL RESPONSES OF SQUIRREL MONKEYS TO $2G_z$ ACCELERATION

C.A. Fuller, J. Tremor*, J.P. Connolly* and B.A. Williams*

Division of Biomedical Sciences, University of California, Riverside, California, 92521;
*Biosystems Division, NASA/AMES Research Center, Moffett Field, CA 94035

This study examines the responses of squirrel monkeys to acute $+2G_z$ exposure. Body temperature responses of loosely restrained animals were recorded via a thermistor in the colon. Behavioral responses were recorded by video monitoring. After baseline recording at 1G, monkeys were exposed to 2G for 60 min. The body temperature started to fall within 10 min of the onset of centrifugation and declined an average of 1.4°C in 60 min. This is in contrast to a stable body temperature during the control period. Further, after a few minutes at 2G, the animals became drowsy and appeared to fall asleep. During the control period, however, they were alert and continually shifting their gaze about the cage. Thus, primates are susceptible to hypergravic fields in the $+G_z$ orientation. The depression in primate body temperature was consistent and significant. Further, the observed drowsiness in this study has significant ramifications regarding alertness and performance in man.

The homeostatic capabilities of animals have been demonstrated to be sensitive to changes in the ambient acceleration environment. Such changes in centrifuged rats and dogs include depressed body temperatures (1,3,4,5), alterations in circadian timekeeping (3,4) and changes in body composition (6). To date, however, little work has been done examining these changes in man or any other primate. Smith et al, (6) was unable to find body composition changes (body temperature was not measured) in a non-human primate (Macaca nemestrina) undergoing cautious chronic centrifugation.

In this study we report that another non-human primate, the squirrel monkey (Saimiri sciureus), is sensitive to acute changes in the acceleration environment. When these animals are exposed to a hyperdynamic field the body temperature was consistently depressed and the animals showed behavioral indications of increased drowsiness.

Four loosely restrained squirrel monkeys were exposed to hyperdynamic fields. The centrifugation consisted of a 60-min step change in the acceleration environment from 1G to 2 G in the z axis (head-to-toe). The animals were allowed to acclimate to the centrifuge for as much time as necessary for the body temperature to stabilize prior to centrifugation (60 to 120 min). Body temperature was measured via a thermistor inserted six centimeters past the anus and taped to the base of the tail. Temperature information was recorded continuously on a strip chart recorder. The data was subsequently digitized at one minute intervals, and average responses were computed. Videotapes were made of the animals' behavioral responses throughout the centrifugation, including control and recovery periods before and after centrifugation.

All animals demonstrated significant depressions in body temperature in the hyperdynamic environment. Figure 1 shows the average response of the four animals to a total of six centrifuge runs. After the centrifuge was started, there was generally a small increase in body temperature. Within 5 to 10 min, however, a continuous decline in temperature became apparent. The average decrease in body temperature was 1.4°C and the depression occurred for the duration of the step change. The shaded area indicates the region ± one standard error around the average temperature response. Approximately five min after the animal was returned to 1G the body temperature began to rise.

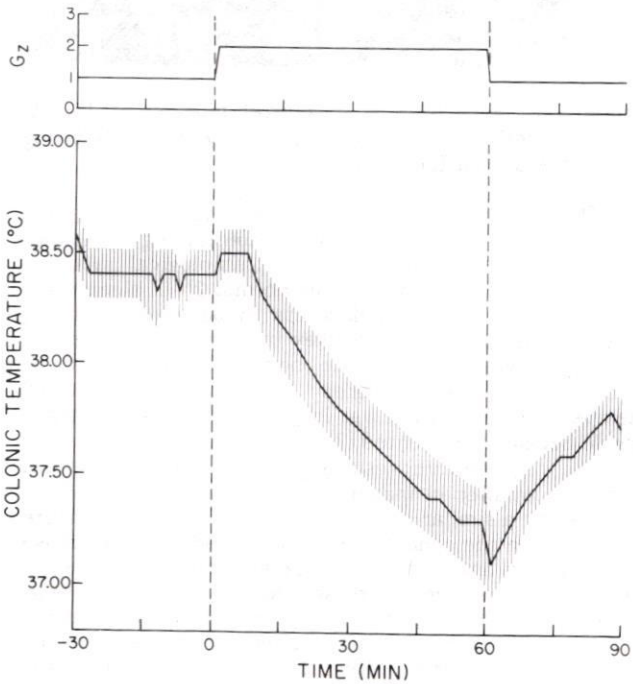


Figure 1. The bottom panel shows the average body temperature response (mean ± standard error) of 4 squirrel monkeys to exposure to 2G for 60 min. The top panel shows the 1G and 2G environments.

Figure 2 shows the typical behaviors of an animal before (A) and during (B) exposure to 2G. Normally, the animals were alert and continually examining their environment before centrifugation. Within a few minutes of the initiation of the 2G environment, the animals consistently tended to show an increased drowsiness as evidenced by visual observation. This behavior often continued well into the 1G environment subsequent to acceleration. The sleeping behavior tended to alternate with periods of arousal, since when the animals relaxed they sank in the restraint system and tended to slip off their perch. Falling aroused the animals at which time they reestablished themselves upon the perch and started to doze off again, repeating the cycle.

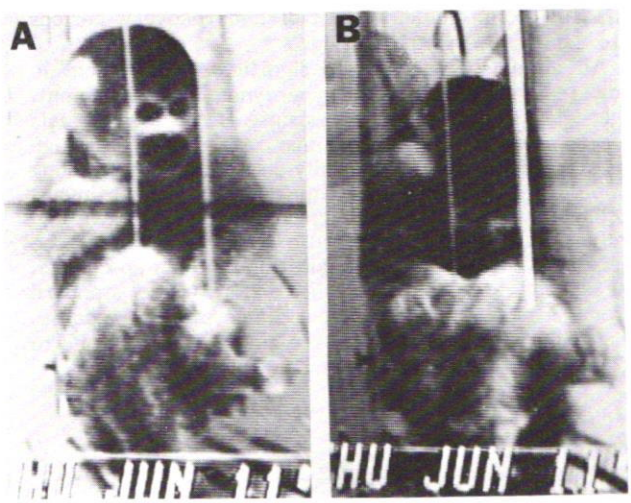


Figure 2. Responses of a squirrel monkey at 1G (A) showing alert behavior and subsequently at 2G (B) during which the animal fell asleep.

The concomitant fall in body temperature and increased sleepiness is appropriate considering the known interactions between these two physiological systems. For example, sleep onset in the normal 24-hr sleep-wake cycle coincides with a decrease in body temperature. Also, in the Biosatellite monkey (the only other instance in which we know primate body temperature to be measured in an altered dynamic environment), temperature regulation was impaired and the sleep-wake cycle became fragmented (2).

The temperature response in the study is similar, although smaller in magnitude, to that seen in rodents exposed to a similar environment (1). These temperature responses in the rodent are accompanied by an increase in heat loss and decrease in oxygen consumption (heat production). However, these specific changes in heat production and heat loss were determined not to be a thermoregulatory response elicited by changes in the temperature control system of the rat. Further, the ability of centrifuged rats (2G) to maintain body temperature in the presence of a cold exposure is markedly impaired. The mechanism eliciting these changes in body temperature are unknown at this point.

One possible mechanism for these responses, which needs further investigation, is related to the orientation of the hyperdynamic environment. Several observations indicate that mechanical pressure on the ventral surface of the brain as a result of acceleration may play a role in this response. Rats centrifuged in an inverted position show a smaller body temperature response (1). The magnitude of the initial decline in body temperature is a function of the intensity of the acceleration field over a range of 1.12G to 2.5G (4). The responses of animals to the hyperdynamic field involve hypothalamically-controlled functions including: 1) temperature regulation, 2) circadian timekeeping, 3) sleep, 4) body composition, 5) cardiovascular responses and 6) feeding behavior. Further, the orientation of the dynamic environment may explain the differences between our study and the study of Smith et al (6), in which no primate response was observed. In the present study the acceleration vector was controlled for the z axis by the loose restraint of the animal, while the macaques in the earlier study were unrestrained and may have adopted a posture which led to a significantly different acceleration vector.

Thus, we have shown that the squirrel monkey is sensitive to acute exposure to $+2G_z$ in at least two physiological systems: temperature regulation and sleep/wake behavior. Such responses indicate that careful consideration must be given to primates and man in the hyperdynamic environment. Further work is needed to define the responses in these and other systems and to determine the mechanisms involved.

REFERENCES

1. Fuller, C.A., J.M. Horowitz and B.A. Horwitz. Effects of acceleration on the thermoregulatory responses of unanesthetized rats. J. Appl. Physiol. 42:74-79, 1977.
2. Hanley, J. and W.R. Adey. Sleep and wake states in the Biosatellite III monkey: Visual and computer analysis of telemetered electroencephalographic data from earth orbital flight. Aerosp. Med. 42: 204-313, 1971.
3. Oyama, J. Response and adaptation of beagle dogs to hypergravity. In: COSPAR Life Sciences and Space Research XIII, (Ed. by P.H.A. Sneath) Akademik-Verlag: Berlin, p. 2-17, 1975.
4. Oyama, J., W.T. Platt, and V.B. Holland. Deep-body temperature changes in rats exposed to chronic centrifugation. Am. J. Physiol. 221: 1217-1277, 1971.
5. Schertel, E.R., J.M. Horowitz and B.A. Horwitz. Effects of gravitational profiles on the rat's thermoregulatory response to cold. J. Appl. Physiol. 49: 663-668, 1980.
6. Smith, A.H., D.F. Rahlmann, A.M. Kodama, and N. Pace. Metabolic responses of monkeys to increased gravitational fields. In: COSPAR Life Sciences and Space Research XII, (Ed. by P.H.A. Sneath) Akademik-Verlag: Berlin, p. 129-132, 1974.

ACKNOWLEDGEMENTS

We thank Ames Research Center for use of its facilities, Jim Black, Richard Takeda and Gretchen Thompson for help in data analysis and Nancy Carwile for preparation of the manuscript. Supported in part by NASA Grant NAS-2-10536, NSF Grant BNS-792441 and PHS Grant BRD-RR09070.

GRAVITY PERCEPTION AND ASYMMETRIC GROWTH IN PLANTS: A MODEL DERIVED FROM THE GRASS PULVINUS

P. Dayanandan, C.I. Franklin and P. B. Kaufman

Department of Botany, University of Michigan, Ann Arbor, Michigan 48109

Abstract. Gravitropic responses in plants involve asymmetric growth. Based on the geometry of growth response in grass leaf sheath pulvinus a general model is proposed for gravitropism in multicellular plant organs. The negative gravitropic response of a plivinus is a consequence of cell elongation involving all but the uppermost region of a horizontally placed organ. The uppermost region does not grow while the lowermost region elongates maximally. The regions inbetween elongate to intermediate extents. The angle of curvature θ of the organ is related to the diameter D and the initial (L_0) and final (L_1) lengths of the organ by the expression $\theta = (L_1 - L_0)/D$ where θ is in radians. The response of the individual cells can be expressed as inherent sensitivity S to gravitational stimulus by the equation $S = 0.5 - r \cos \theta$ where r is the radius of the organ and θ the angle of curvature in degrees. Among the inferences derived from this model are: the importance of cellular asymmetry as a basis for organ asymmetry; a fundamental distinction between the gravitropic responses of unicellular and multicellular structures; and difficulties in accepting lateral transport of hormone as a basis for gravitropic response.

INTRODUCTION

The perception of gravitational stimulus in plants reoriented from their normal vertical positions leads to differential growth resulting in curvature. The geometry of the growth response was first analysed by Sachs (1). However, the importance of the differential growth that involves every cell of a gravitropic organ has not been fully appreciated. Instead, most workers have assumed that curvature is brought about merely by elongation of the lower surface (in a negatively gravitropic organ) or the upper surface (in a positively gravitropic organ). This gross oversimplification has also resulted in the ready acceptance of lateral hormone transport as the cause of growth responses. Our analysis of the geometry of growth response in grass leaf sheath pulvinus reveals a precisely controlled growth response that resides at the cellular level. Every cell seems to "recognize" its position in relation to every other cell of the organ in the gravity vector. We propose a model that has equal applicability to all multicellular organs that respond to gravity.

METHODS

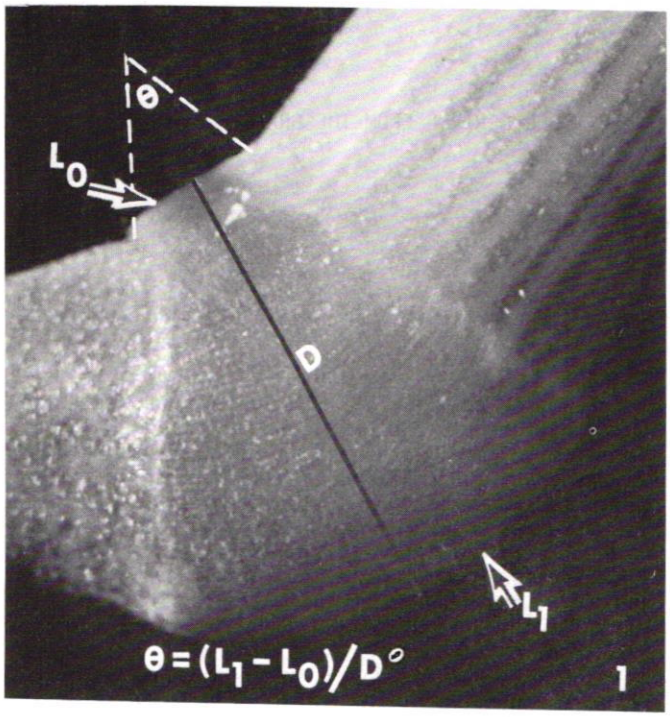
Leaf sheath pulvini located adjacent to the nodal regions of oats (Avena sativa) and barley (Hordeum vulgare) were used in this study. Details of the plant material and methods of treatment were previously described (2). The geometry of growth response was studied by photographing the responding pulivini at different time intervals. Cellular responses were studied by measuring the isolated epidermal peels and sectioned material.

Segments were also split lengthwise to isolate two identical halves. Split segments were placed horizontally but rotated to orient the half-pulvini at different angles in relation to the gravity vector. Such orientations resulted in positioning the intact outer epidermis facing downwards, upwards or in several intermediate angles. The effects of a number of growth promoting or inhibiting substances were also studied by supplying such chemicals dissolved in 0.1 M sucrose to split pulvini in horizontal positions.

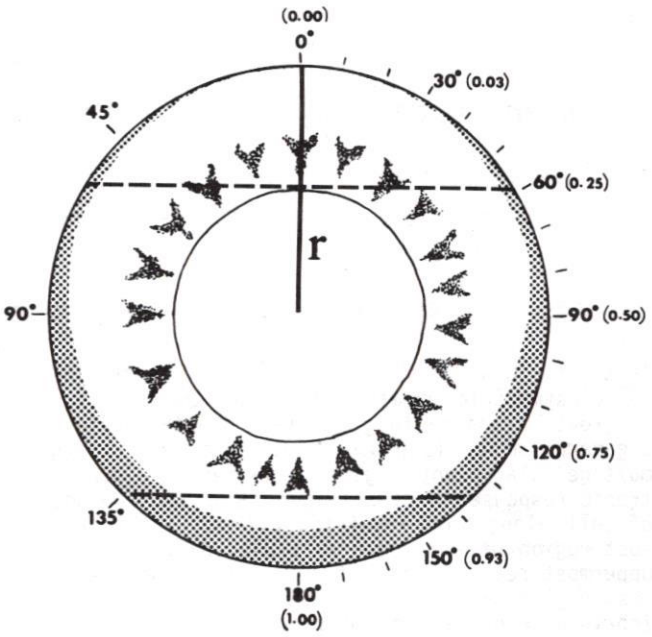
RESULTS

Figure 1 is a photograph of an oats segment that has curved to 52°. Growth response is confined to the pulvinus. As seen in this photograph, the response of the pulvinus is linear with no growth on the upper surface to maximum growth on the lower surface. Since a cylindrical organ is being viewed from one side, the half-circumferential distance between the upper and the lower sides represents the diameter D, of the pulvinus. L_0 represents the initial length of the pulvinus which has not changed on the upper surface. L_1 is the maximal length seen on the lower side. When two lines are drawn along the boundaries of the pulvinus and allowed to meet on the upper side, and angle θ is generated. The value of this angle is the same as the angle of curvature of the segment. Since the lower and upper surfaces of the pulvinus correspond to the outer and inner arcs of a cricle whose centre lies where the dashed lines meet, it is possible to establish a relationship between these parameters. Such a relationship is given by the equation $\theta = (L_1 - L_0)/D$ where θ is in radians.

This equation is applicable to all organs that respond to gravity either positively or negatively. Contraction or some elongation on the upper side (L_0) leading to changes in the initial length can also be accomodated with appropriate alteration of L_0. This equation makes it clear why it is that the magnitude of growth responses in different cells has not been easy to analyze. The difference



1



GRAVITY-SENSITIVITY PROFILE

$S = 0.5 - r\cos\theta$

2

in length between the initial and final sizes is a function of the initial length for any given angle and diameter.

Figure 2 is a diagrammatic representation of a cross section of a pulvinus. The upper and lower surfaces are here indicated by the 0° and 180° positions respectively. The responses of the cells in the periphery are shown by values in brackets. The upper surface has no response while the lower surface has a maximum of 1 whereas the mid-point (90° position) has a value of 0.5. The shaded area along the inner periphery is a diagrammatic representation of the relative responses of the cells in the periphery. The dashed lines are two representative isobars indicating identical elongation of cells inside the pulvinus.

Based on the trigonometric relationship that exists between the different positions shown in Figure 2 we have derived an equation that describes the sensitivity of every cell in the organ to gravitropic stimulus. The sensitivity $S = 0.5 - r \cos \theta$ where r is the radius and θ is in degrees. When r itself is 0.5 the equation describes the sensitivity of the epidermal layer. When r is 0.25 the equation describes the sensitivity of cells lying halfway from the centre of the circle. Thus, by varying the value of r the sensitivity of any cell can be determined.

The inner circle of wedge-shaped stipled areas indicate the location of the statenchyma. If statenchyma is involved in the release of IAA as is often assumed, it is difficult to explain the growth response of cells well above the level of statechyma.

The differential growth response of the organ is a consequence of the differential growth responses of the individual cells. In a vertical organ, the cells are distributed in a radially symmetrical manner. On horizontal displacement, asymmetry is established. This organ asymmetry could reflect a preformed cellular asymmetry that gets activated after gravity stimulation. In unicellular structures, such as Chara rhizoids a preexisting cellular asymmetry in not a requirement for the bending response. Asymmetry in such cells can be achieved as a consequence of horizontal displacement alone.

Experiments with split segments oriented in different positions show that individual segments can bend as much as the intact organ. Depression in the curvature of a segment can be caused by growth inhibitors as well as growth promotors. Inhibitors of protein and RNA synthesis prevent any growth response in the pulvinus while a compound like indoleacetonitrile causes growth of the pulvinus but depresses curvature by destroying the asymmetry of growth. It is customary to half the gravitropic organs to analyze for growth promoting substances. It should be remembered that the two halves are not equivalent to regions of no growth and positive growth. Growth is present throughout the entire organ.

REFERENCES

1. Sachs, J. 1887. Geotropism and heliotropism. In: Lectures on the physiology of plants. Clarendon, Oxford.
2. Dayanandan, P., F.V. Hebard, and P.B. Kaufman. 1976. Cell elongation in the grass pulvinus in response to geotropic stimulation and auxin application. Planta 131: 245-252.

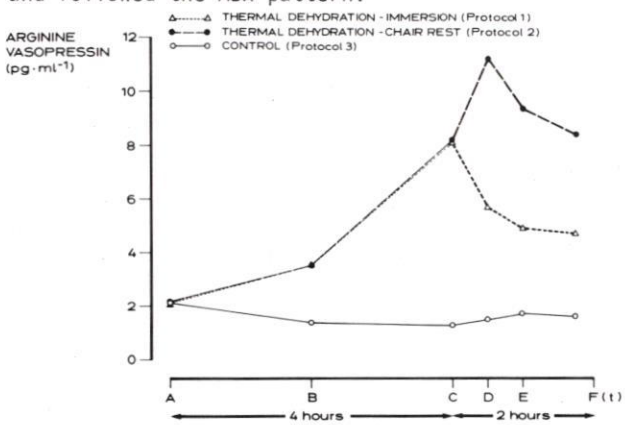
This research was supported by grant NAGW-34 from the National Aeronautics and Space Administration.

ADH suppression under immersion combined with dehydration

H.von Ameln, M.Laniado, L.Röcker,K.Kirsch,H.J.Wicke, I.Busch

Free University of Berlin, 1000 Berlin 33, Germany

It can be assumed that under zero gravity an increased central blood volume goes hand in hand with an elevated plasma osmolality. This creates a conflicting situation with respect to the ADH secretion. According to Verney (1), an increased plasma osmolality stimulates ADH secretion. On the other hand, as proposed by Gauer and Henry (2) an increment of central blood volume suppresses ADH secretion. We investigated this situation by application of the immersion model in dehydrated subjects and followed the ADH pattern.



Each subject underwent three different protocols. After a standardized breakfast, the subjects layed down for 1 h in supine position. Afterwards the first blood sample was taken (A). According to protocol 1, this was followed by a 4 h thermal dehydration period in a heat chamber. 2 h after the onset and at the end of the thermal dehydration period blood samples B and C were taken. Immediately thereafter, 2 h of head out water immersion followed. 3 additional blood samples were taken during that time: sample D 30 min, sample E 1 h, and sample F 2 h after starting the immersion. The same subjects followed protocol 2 about 8 days later. Until the end of the thermal dehydration period the experimental procedure was identical with that explained above. However, instead of an immersion period the subjects rested on a chair for 2 h. Another 8 days later, the subjects rested for 7 h in supine position at room temperature (protocol 3). Blood sample analysis were done for plasma osmolality, serum sodium and plasma ADH.

Results: According to protocol 1 and 2, body weight had decreased by about 3.5% at the end of the thermal dehydration period. Only a small body weight reduction occurred during the following immersion period as well as during the chair rest period. Plasma osmolality and serum sodium increased significantly from 288 to 292 mOsmol/l and 137.5 to 139.0 mmol/l respectively under thermal dehydration. Thereafter, during immersion and during chair rest they furthermore increased slightly. In protocol 3 no noteworthy changes occurred.

Under the assumption that the body weight reduction is due to mainly a water loss, one can conclude that 4 h of heat exposure resulted in a hypertonic dehydration. Both an increased plasma osmolality and a hypovolemia are known to stimulate ADH secretion. This can be seen from the figure. In the figure the ADH concentration is followed over the period of the protocols. The diagram shows the expected increase in plasma ADH concentration during the thermal dehydration periods (A-C). Under both, protocol 1 and 2, ADH levels rose from 2 pg/ml to 8 pg/ml, whereas following protocol 2 ADH remained unchanged.

Head out water immersion leads to a volume shift of about 700 ml into the intrathoracic compartment. Therefore, in a situation characterized by a high plasma osmolality combined with a hypovolemia at the end of the thermal dehydration period the maximal stimulatory drive for ADH secretion should be counteracted by the immersion. This can be seen from the right part of the figure (D-F). Whereas during chair rest (protocol 2) the ADH concentration remained high or even increased, a pronounced drop from 8 pg/ml to 4 pg/ml is visible under immersion. This drop of ADH concentration occurs in face of an elevated plasma osmolality. In all likelihood this is due to the stimulation of intrathoracic stretch receptors. The fact that the ADH concentration is still above the control levels can be related to the osmostimulus whereas the difference between protocols 2 and 1 accounts for the volume stimulus. Therefore, our experiments allowed to separate the influences of plasma osmolality and blood volume on ADH secretion.

REFERENCES

1. Verney,E.B. 1947. The antidiuretic hormone and the factors which determine its release. Proc.Roy.Soc. B 135:25-106.
2. Gauer,O.H. and J.P.Henry. 1963. Circulatory basis of fluid volume control. Physiol.Rev. 43:423-481.

33rd Annual Fall Meeting
of the
AMERICAN PHYSIOLOGICAL SOCIETY

With

DIVISION OF COMPARATIVE PHYSIOLOGY AND BIOCHEMISTRY OF THE AMERICAN SOCIETY OF ZOOLOGISTS

LATIN AMERICAN ASSOCIATION OF PHYSIOLOGICAL SCIENCES (ALACF)

IUPS COMMISSION ON GRAVITATIONAL PHYSIOLOGY

Town & Country Hotel
San Diego, California

October 10-15, 1982

Scientific Program

REFRESHER COURSE:

Microcirculation

SYMPOSIA:

Blood Brain Barrier
Compartmentation and Exchange of Calcium in the Heart (2 sessions)
Is Efferent Control of Arterial Baroreceptors Important?
Regional Vascular Behavior in the Gastrointestinal Wall
Temperature Effects on Fish
Anaerobic Energy Metabolism of Invertebrates
Man at High Altitude (3 sessions)
Ionic Channels in Excitable Membranes
Differentiation of Epithelial Cells
Gravitational Physiology
Neurophysiological Mechanisms Controlling Circadian Rhythmicity

TUTORIAL LECTURES (Tentative)

Extrinsic and Intrinsic Control of Secretion
Water Channels in Red Blood Cells
Hormonal Control of Fetal and Neonatal Growth
New Concepts in Acid-Base Balance
Neurochemical Mechanisms of Thermoregulation
Thermoregulation During Exercise
New Concepts of Nephron Structure
The Role of Aldosterone, Sodium, Chloride and Potassium in Metabolic Alkalosis
Glomerular Filtration
Pattern Generators in the Central Nervous System of Mammals
Neural Integration at the Level of Autonomic Ganglia
Calcium Regulations in Osteoporsis
Neural Regulation of Ideal Smooth Muscles
Membrane Transport Processes
Endorphins
Membrane Structure and Function
Comparative Reproductive Physiology
Neuronal Integration
Cerebral Cortex
Physiology of Bile

Call for Abstracts to be Mailed in March, 1982
Deadline for Receipt of Abstracts, June 11, 1982
Deadline for Advance Registration, August 27, 1982

The 33rd Annual Fall Meeting of the American Physiological Society will be held October 10-15, 1982, at the Town & Country Hotel, San Diego, California. Three other physiological groups will meet with APS in 1982: the Division of Comparative Physiology and Biochemistry of the American Society of Zoologists; Latin American Association of Physiological Sciences (ALACF); and the IUPS Commission on Gravitational Physiology.

The Call for Abstracts including general information, housing forms, and registration cards will be mailed to members of all participating groups in March, 1982. Nonmembers who wish to receive the mailing should complete the information requested below and return to the APS Fall Meeting Office, 9650 Rockville Pike, Bethesda, MD 20814.

The APS Refresher Course will be conducted on Monday, October 11, the Bowditch Lecture will take place on Tuesday, October 12, and the APS Business Meeting on Thursday, October 14. Scientific sessions will be scheduled from 9:00 AM to 4:30 PM Tuesday through Friday. Each half day will consist of one or more symposia, tutorial lectures, and simultaneous sessions of volunteer papers presented either in slide or poster sessions.

We invite all those interested in the physiological sciences to participate in the meeting.

REQUEST FOR FORMS – 1982 APS FALL MEETING

Deadline for receipt of Abstracts: **Friday, June 11, 1982**

	Quantity
Abstract sets	_____
Information leaflet	_____
Advance registration card	_____
Hotel reservation form	_____
Placement Service Information	_____

Candidate _____ Employer _____

→ → → → → → → → → →
Please print your name and complete mailing address with Zip Code
→ → → → → → → → → →

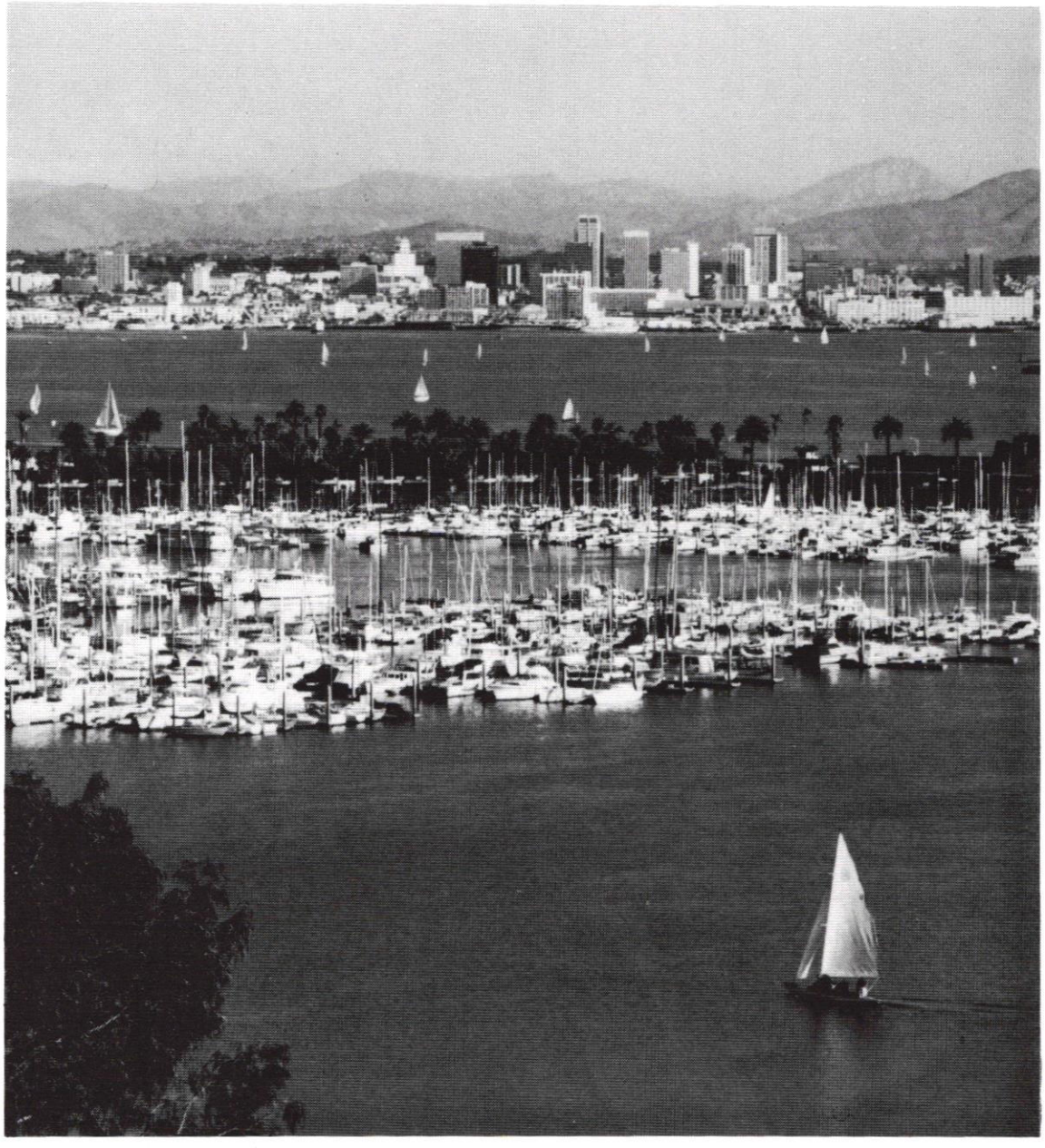
Welcome to America's Finest City

San Diego—a near-perfect climate graces the inviting environment year-round; the Pacific Ocean washes miles of sun-baked beaches. And the people take unique pride in keeping the City's majestic beauty unspoiled.

San Diego offers an exciting variety of pleasurable experiences. There's Sea World, world-famous San Diego Zoo, the fantastic new Wild Animal Park, 69 golf courses, every conceivable water sport, horse racing and major league sports. And don't forget San Diego's sumptuous dining—from Continental to Polynesian to California restaurants.

The color and thrills of enchanting Mexico lie just 30 minutes to the south.

This is San Diego, a world with so much to discover. So much to enjoy.



THE NASA SPACE BIOLOGY GRANT PROGRAM

The objective of NASA's Space Biology research program has been to use gravity to solve relevant terrestrial biological problems and to enhance our capability to use and explore space. The advent of the space age provided the first access to the 'gravity-free' state and an opportunity to manipulate gravity from its norm of one down to zero. Therefore, NASA has assumed the responsibility to investigate the biological significance of gravity and thereby expand biological knowledge.

Goals

The goals of the Space Biology program are:

1. To enhance our knowledge of normal physiological adaptive mechanisms in both plants and animals and thereby provide new insight into both normal and pathological mechanisms.

2. To provide for the multiple generation survival of plants and animals in space through an understanding -- and ultimately control -- of the effects of gravity on development, adaptation and evolution.

3. To enhance plant productivity through an understanding and control of gravitational and related environmental stimuli and the manipulation of response mechanisms.

The achievement of such goals depend upon answers to basic scientific questions that include the following:

1. Does gravity influence fertilization and early development, and can fertilization and early development proceed normally in a near 0-g environment? If gravity does affect fertilization and early development, what are the sensitive physiological systems and how are they affected? If early development is affected by gravity, is it a result of an effect on the parent or the direct effect on the embryo itself?

2. What is the role of gravity in the formation of structural elements such as lignin, cellulose, chitin and bone-calcium at the molecular as well as at the more complex organizational levels?

3. What role does gravity play in Ca-mediated physiological mechanisms and in Ca metabolism?

4. What is the gravity sensing mechanism? How does it perceive information? How is the information transmitted to evoke a response?

5. How does gravity as an environmental factor interact with other environmental factors to control the physiology, morphology and behavior of organisms? Or how do gravitational and other environmental stimuli interact in their control and direction of living forms? Can the action of gravity be replaced by different stimuli?

Strategy

The strategy so far has been to manipulate gravity on earth and develop weightless simulation to: (a) develop and test gravitational hypotheses, (b) identify gravity sensitive biological systems and interacting environmental response mechanisms, (c) address valid gravitational biological questions on earth when possible, and (d) plan and design future space experiments. As longer flight missions, both manned and unmanned, become available more emphasis will be placed on flight experiments. Biological questions that require longer periods of micro-gravity will also be stressed.

Program Content

The program has been divided into the following three broad areas:

1. The role of gravity in development, maturation, senescence and evolution.

2. Gravity receptor mechanisms. These include the identification of the organ or site of gravity reception and the biological systems and mechanisms that transmit the information to a responsive site.

3. The physiological effects of gravity. This includes the biological mechanisms by which living systems respond and adapt to altered gravity, particularly that of the space environment, as well as the interactive effects of gravity and other stimuli and stresses on the physiology of organisms.

This NASA Program in space biology is carried out intramurally by the NASA Research Centers and by a system of extramural grants. Qualified scientists interested in learning more about the program and the development of research proposals should contact:

Dr. Thora W. Halstead
Chief, Space Biology Program
Life Sciences Division
NASA Headquarters
Washington, D.C. 20546