Korean J Biol Sci 4: 231-237, 2000

Effects of Microgravity and Hypergravity on Aging and Longevity of Insects

Hak Ryul Kim

Department of Biology, College of Sciences, Korea University, Seoul 136-701, Korea

Key Words:
Aging
Drosophila melanogaster
Hypergravity
Insect
Longevity
Microgravity

The effects of microgravity and hypergravity on aging are still poorly documented, particularly in mammals. However, there is a growing interest for the use of the fruit fly, *Drosophila melanogaster*, and this species may be now considered as a model organism in gravitational biology studies dealing with aging.

The observation of bone demineralization and muscle atrophy in the first astronauts and cosmonauts was considered by some authors to support the idea that some organ systems may age faster in weightlessness conditions (Miquel & Economos, 1982). In fact, with the occurrence of spaceflights, gravity now is thought to be a variable affecting life processes.

Since spaceflights devoted to biology experiments are scarce and expensive, hypergravity (HG) has been considered by some authors to be a useful way to study effects of gravity on the aging process. Furthermore, it has been emphasized that the data obtained during HG experiments are insufficiently used in analyzing and predicting effects of microgravity (μG) (Serova et al., 1985). Relying on the rate of living theory (Pearl, 1928), which states that increased metabolism results in increased rate of aging and decreased longevity, the gerontologists of NASA considered that, due to the weight increase in HG, metabolism should increase in HG and, therefore, that longevity should decrease (Economos, 1982). On the other hand, it was expected that in microgravity between 0 and 1 *g*, the rate of aging is decreased and longevity extended (Economos, 1982).

Microgravity and Aging

Longevity

No subject of any species has lived for its entire life span in μG. This is obviously due both to the short duration of many spaceflights and to problems linked to maintenance of animals for their entire life in μG.

Miquel et al. (1978) embarked approximately 400 *D. melanogaster* (4-day-old larvae) aboard the Soviet Cosmos 782 biosatellite for a flight of 19.5 d (Fig. 1, top). One half of the flies was kept at 1 *g*, by using an on-board centrifuge, and the other half lived in μG. In both sexes, no gravity-linked longevity difference was observed in these flies which lived for about 100 d (starting from egg-laying and not from emergence). These flies thus spent about 20% of their life in the space environment. Unfortunately, as the authors did not report the longevity of their 1-*g* ground control groups, it is impossible to know whether the features of the spaceflight had some effect on longevity.

Using a different strain from that used in Cosmos 782, Miquel and Philpott (1978) embarked 400 male flies (larvae and young and middle-aged adults, respectively, 7 and 26 d old) aboard Cosmos 936 for a 18.5 d-flight, but without an on-board 1-*g* centrifuge (Fig. 1, middle). On one hand, the flies embarked as young adults lived for a shorter time than 1-*g* ground controls (mean±SEM, 59±1.2 vs. 84±1.3 d, ages starting from emergence). On the other hand, the life span of flies embarked as middle-aged adults was not much lower than that of their 1-*g* ground controls (83 ±1.3 vs. 91±1.4 d). It seems difficult to argue that the low longevity of the flies embarked as young adults was due to μG itself, considering the longevity difference with the flies embarked at middle age and the absence of gravity effect in Cosmos 782, where a 1-*g* centrifuge was used. The longevity difference between the flies embarked as young adults aboard Cosmos 936 and their respective controls could be due to other features of the space environment (e.g., cosmic radiations or dynamic factors of the flight). Nevertheless, it has to be kept in mind that the longevity of the flies embarked as middle-aged adults aboard Cosmos 936 was not very different from that of their 1-*g* ground controls.

Lee et al. (1985) have reported longevity results of houseflies which emerged the first day of a 7-day U.S. space shuttle flight. No difference was observed between

*To whom correspondence should be addressed.
Tel: 82-2-3290-3155, Fax: 82-2-924-6094
E-mail: kimhr@kuccnx.korea.ac.kr

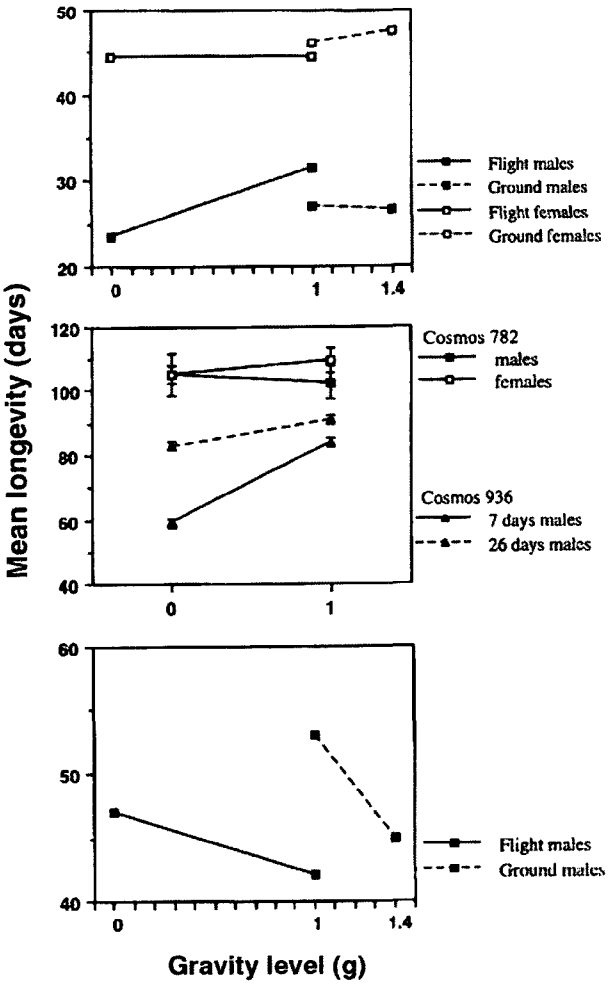

Fig. 1. Effects of µG on longevity in *D. melanogaster* flies. Top: *n* are not given in the paper of Marco et al. (1986). Middle: *n*=30 for each group of males and between 50 and 60 for groups of females in Cosmos 782; *n* varies between 70 and 85 for each group of sex and gravity in Cosmos 936. Bottom: *n* are not given in the paper of Benguria et al. (1996).

these µG flies and their 1-*g* ground controls, but the authors themselves emphasized that sample sizes were low (10 flies in the shuttle and 12 on ground). Marco et al. (Marco et al., 1986) submitted *D. melanogaster* flies to a 7-day U.S. space shuttle flight (Fig. 1, top). The longevity of females was about 45 d in all groups. Microgravity male flies lived shorter than 1-*g* flown flies and shorter than 1-*g* and 1.4-*g* Earth controls. However, it is impossible make a conclusion from these results, because sample sizes and statistical analysis were not provided.

Benguria et al. (1996) recorded the longevity of adult flies which have flown for 14 d within a space shuttle (Fig. 1, bottom). The authors reported that 0-*g* females had the same longevity curve as 1-*g* ground females, but they did not report the data of the 1-*g* flown females and 1.4-*g* ground females. Furthermore, they did not provide the mean longevity of any group. This may be understood because it was impossible to compute these data for males, because the authors sacrificed some of the flies used in the longevity experiment: all surviving males were killed at Day 54 when the percents of survival reached 50% and 30% of the initial sample of 0-*g* flies and 1-*g* ground flies, respectively, Nevertheless, the authors provided longevity curves for these flies, up to Day 53 of life, and indicated that 0-*g* flies survived for a shorter time than 1-*g* ground flies; from the published curves, the median longevity of the 0-*g* group was about 47 d, but about 53 d in the other group. This result allowed the authors to conclude that longevity decreased in µG. However, when one compares the curve of 0-*g* males with that of the 1-*g* males embarked aboard the shuttle, the inescapable conclusion is that the median longevity of this last group was about 42 d. In other words, when one compares the two groups of embarked flies, 1-*g* flies lived shorter than 0-*g* flies, a result not discussed by the authors. This result also shows that the two 1-*g* groups kept either on the ground or within the shuttle had very different median longevities, 42 d and 53 d respectively, Because these control groups were so different, it is somewhat premature to conclude anything about the effect of µG on lifespan.

It is very frustrating to conclude this review by stating that, for the time being, properly designed experiments have not been carried out or, if properly designed, poor analyses of data have been given. As it was in the early 1970s, the effect of µG on longevity is still not well understood. We hope that authors will pay more attention to these drawbacks in future studies and will give new and good results on the matter.

Aging

Studying aging in a permanent µG environment is a challenge. Obviously, it is currently difficult to imagine performing measurement of, e.g., the activity of various enzymes or any other biochemical index, during an orbital flight. In the same way, time constraints of the crew prohibit performing time-consuming experiments, such as daily behavioral observations for many days. In such conditions, most of experiments follow the same basic procedure: animals are embarked aboard a spacecraft and, after return to Earth, their behavior, morphology, and other features are observed. In other words, these experiments do not investigate the effect of µG but the aftereffects of exposure to space environment factors, one of them being µG. It is worth noting, however, that experiments dealing with developmental biology have been done during orbital flights (Bucker et al., 1986 and Souza et al., 1995).

Miquel et al. (1978) embarked approximately 400 *D. melanogaster* (4-day-old larvae) aboard the Soviet Cosmos 782 biosatellite for a flight of 19.5 d. One half of the flies was kept at 1 *g*, by using an on-board centrifuge, and the other half lived in µG. The body

weight was measured 1 wk after retrieval, at 33 d of age (from egg-laying), in these flies and in two 1-*g* ground controls (standard lab conditions-lab control-and conditions mimicking the satellite flies-synchronous control). The authors reported no difference between groups of females. In males, the body weight difference between the two ground controls was higher than that between the two Cosmos groups, the latter being not significant. In addition to this absence of gravity-related difference, external morphology, fine structure of wing muscle, lipofuscin accumulation, or glycogen content did not vary between the groups. Climbing activity, defined as the "percent of flies able to reach the 250 ml mark in a volumetric cylinder in 20 s (mean of 10 consecutive trials)," was measured in groups of 20-50 65-day-old flies after manual shaking to the bottom of the vial. Eighty-five percent of synchronous control flies, 73% of 1-*g* cosmos flies and 78% of and 0-*g* Cosmos flies reached the 250 ml mark. It is difficult to conclude about the significance of the results because no statistical analysis was given, but these percentages are rather similar. However, some differences were noticed in mating competence, which was observed 3 d before the climbing experiment. In a total of 20 flies, all synchronous controls 19 1-*g* Cosmos flies and 15-*g* flies were able to mate; unfortunately, the expected values are too low to safely compute a 2 statistic. The latency to the first mating was lower in the synchronous controls (6.6 min, SEM unknown) than in the Cosmos groups, which did not differ from each other (1 *g*, 12.1 min; 0 *g*, 11.6 min). Here again however, no statistical analysis was performed. It should be noticed that no difference was observed between the 0-*g* and 1-*g* Cosmos groups. In summary, the Cosmos 782 experiment did not show any effect of μG on various characters, whether the characters were measured 1 wk (body weight), 2 wk (glycogen content), or about 1 month (mating and climbing abilities) after retrieval.

After this experiment was done, Miquel and Philpott (1978) embarked 400 male *D. melanogaster* flies (larvae, young, and middle-aged adults) aboard Cosmos 936 for an 18.5-day flight, but without an on-board 1-*g* centrifuge. The authors reported that μG flies were lighter than 1-*g* ground controls, but no statistical analysis was done. On the other hand, they did not observe morphological and histological differences between groups, except for the observation of wing injuries in some flown flies (here again, no quantitative data). Mating and climbing abilities were also observed. However, because the procedures used were totally different from those used for Cosmos 782 and no statistical analysis was done, it is difficult to compare the results of the two Cosmos experiments. With regard to climbing activity, 1% of flies embarked as young regard (n=66, one trial only) at 48 d of age (starting from egg-laying), and 38% of ground controls tested at 44 d (n=80) were able to reach the 200 ml

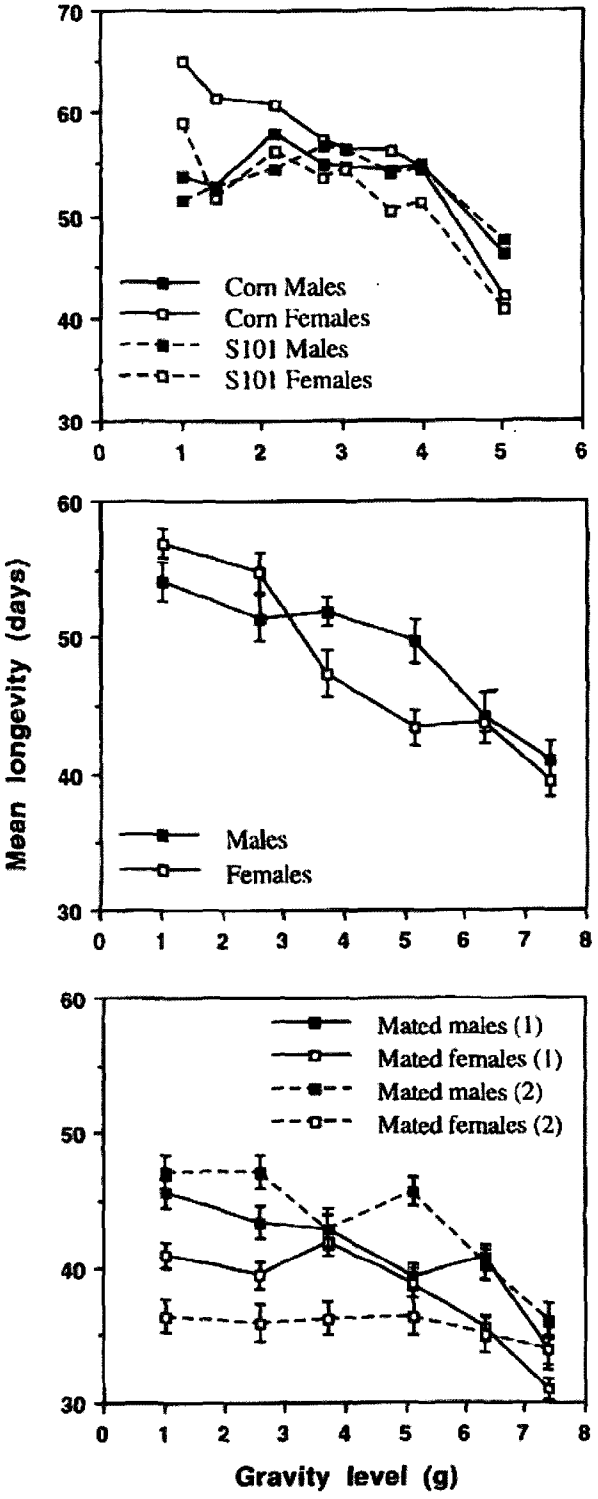

Fig. 2. Effects of HG on longevity in *D. melanogaster* flies. Top: Results of virgin flies in an experiment with a 1- to 5.02-*g* range; for the sake of clarity, SEM have been omitted (SEM range: 0.66-2.18 d); *n*=45 for each group of sex and gravity level. Middle: Results of virgin flies in an experiment using a 1- to 7.38-*g* range; *n*=50 for each group of sex and gravity. Bottom: Results of mated flies in two replicate experiments (1 and 2) by using a 1- to 7.38-*g* range; 2 months separated the two experiments; replicate 1: *n*=100 for each group of sex and gravity level; replicate 2: *n*=50 for each group of sex and gravity.

mark in a 250 ml vial. In the same way, 5% of flies embarked at middle age at 67 d of age (n=71), and 34% of 63 d old ground controls (n=79) reached the mark. The fact that no difference was observed either between the 0- and 1-*g* groups kept in Cosmos 782, or between these flies and the ground control, prohibits the conclusion that the differences observed in Cosmos 936 were due to µG. Regarding mating ability, the published data do not allow a conclusion regarding a difference between Cosmos 936 flies and their ground controls.

Benguria et al. (1996) video-recorded, the locomotor activity of young adult male flies kept at 0 *g* and at 1 *g* in the shuttle every other day during the flight of a shuttle starting 2 d after launch. These data are linked to the longevity results previously reviewed because, in accordance with the Pearl's rate of living theory (Pearl, 1928), the authors considered that increased activity may provoke a decreased longevity. The authors observed motions of flies during a period of one-sixth of a second in containers containing 50 flies at the beginning of the flight, i.e., they observed locomotor activity modulated by reactivity to other flies (see Ewing, 1963). This reactivity probably differed from one container to another, because both the space available to move and the actual number of flies differed between containers (see Fig. 2 in Benguria et al., 1996). As the authors did not perform any detailed analysis of their results but only reported limited data, it is difficult to conclude anything about these results. In the same study, it was reported that the 0-*g* flies, which were considered to be more active than the 1-*g* ground flies, had a higher metabolism. This conclusion was only based on the weight of the feeding trays, inserted within the containers, which was measured after the flight; this weight was lower for the 0-*g* flies than for the 1-*g* ground flies which appears to be not significant. However, the authors did not notice that the weight of the trays of the other 1-*g* group (flies kept in the shuttle) was lower than that of the 0-*g* flies. Mating ability was observed on the ground after the flight at 15, 23, 41, and 53 d of age in the 0-*g* and 1-*g* ground males: the ability of the 0-*g* flies was always lower. However, the 0-*g* and 1-*g* files (n=10) had a mean ($\pm$ SEM) of 1.8 ± 0.13 and 2.0 ± 0.21 copulas in a 1-h period at 15 d of age (n=10), a difference reported to be significant at the ($p < 0.001$) level; given the reported SEM, it seems to be too high. The climbing activity was measured in the same groups at 15, 23, and 41 d of age. The activity of 0-*g* flies was always lower than that of 1-*g* ground flies, but the scores did not clearly decrease with age (see Le Bourg & Lints, 1992a), and the authors considered $P < 0.11$ to be significant.

As for the effects of µG on longevity, there is a paucity of data on aging. Only a few experiments have been carried out and the published reports are very imprecise. It seems clear that future studies have to be well designed and well analysed, in order to get a clear information on µG effects on aging.

Hypergravity and Aging

Longevity

Because the weight increases in HG, a reasonable rationale is that metabolism should also increase in HG and, relying on the rate of living theory (Pearl, 1928), it may be expected that longevity decreases in HG. Before focussing on longevity results, we may wonder whether metabolism does increase in HG.

Indirect measurement of metabolism was also performed in HG-kept *D. melanogaster* females. The fecundity pattern of females kept at various gravity levels throughout life (1-5 *g*) was roughly similar to that of food-restricted females (Lints & Le Bourg, 1989); in other words, females faced with an increased metabolic demand-HG-managed their laying activity like females faced with a decreased metabolic supply-food restriction. This result seems to point out that the metabolic rate is increased in HG and that animals try to adapt to this by modulating fecundity (flies).

Le Bourg and Lints (1992) observed longevity of *D. melanogaster* virgin flies living at different gravity levels throughout life (1, 1.41, 2.16, 2.75, 3.02, 3.61, 3.97, and 5.02 *g*). Two rearing media fit to *Drosophila* needs were used, the usual corn medium (Corn groups on Fig. 2, top) and the S-101 medium of Pearl et al. (Pearl et al., 1926) (S-101 groups on Fig. 2, top), both supplemented with live yeast. The rationale was that the less nutritious medium (S-101) could be deleterious to flies submitted to a high metabolic demand due to their increased weight in HG. As expected, flies lived shorter on the S-101 medium than on the Corn medium, the effect more important in females than in males. The lowest longevities were observed in both sexes and media at the highest gravity level, the males outliving the females. At 1 *g*, the females lived longer than males, the difference between sexes being progressively abolished and reversed as gravity was raised from 1 to 5 *g*. However, contrary to what was expected, the effect of the S-101 condition was not reinforced by gravity and the percentage of stuck flies was very low on the two media (both sexes and all gravity groups pooled: 4.70% on CORN and 4.49% on S-101). The main conclusion of this study was that no clear HG effect was observed in the 1- to 4-*g* range. However, longevity decreased in the flies living at 5 *g* by around 1 wk in males and 3 wk in females, the mean longevity in both sexes remaining at a high value of 45 d. These results are not in accordance with the rate of living theory because, in the two media conditions, a clear longevity decrease was only observed only above 4 *g*. Concerning the effect observed at 5 *g*, one may wonder whether, above 5 *g*, a regular or an abrupt decrease in lifespan would be observed.

Testing HG levels superior to 5 *g* was done in a later experiment (Lints et al., 1993), conducted in somewhat different conditions (another centrifuge: 1, 2.58, 3.70, 5.14, 6.31, 7.38 *g*; another lab; and so on). A slight longevity decrease was observed for virgin males kept on the Corn medium in the 1- to 5.14-*g* range, with a more significant decrease obtained in the 5.14- to 7.38-*g* range (Fig. 2, middle). The effect of HG was more salient for virgin females in the 1- to 5.14-*g* range. At 7.38 *g*, both sexes had roughly the same longevity of 40 d.

In the same experiment, effect of mating on the longevity of flies living in HG was tested; the lifespan of male and female flies, kept together throughout life, was measured (Fig. 2, bottom). As usually observed, mated flies had, from 1 *g* on, a lifespan considerably shorter than virgin flies. An effect of HG on mated flies was observed only in the 5.14- to 7.38-*g* range, which shows that, in a large HG range, HG does not reinforce the effect due to mating. This last result is similar to what was observed with the S-101 rearing medium.

To summarize this set of experiments, we may conclude that, both in virgin and mated flies, a clear longevity decrease was observed mainly above 4 *g*, which means that a linear relationship between gravity level and longevity does not exist, a result not in accordance with the rate of living theory. Furthermore, the effect of HG on longevity of mated flies was less important than that observed for virgin flies. It seems that, even if clear effects of HG on metabolism do exist, longevity is not clearly affected by this increased metabolism.

After the completion of the previous experiments, the effect on longevity of a shorter exposure to HG was investigated (Le Bourg & Minois, 1997). The rationale for this experiment was that mild stresses may have positive effects on longevity, as it has been suggested by other authors (Frolkis, 1993), though too strong stressors are known to be deleterious; a lifetime exposure to HG is perhaps a strong stressor while a shorter exposure could be a mild stress. To test this hypothesis, flies were kept in HG (3 *g* or 5 *g*) for 2 wk from the second day of imaginal life. No gravity effect was observed in females, whereas the HG males lived 10 d longer than the 1-*g* males (Fig. 3). HG is not the only mild stress slightly increasing longevity, because male flies submitted to a short heat shock at young age live 2 d longer than control males, whereas no clear effect is observed in females (Khazaeli et al., 1997).

Aging

In *D. melanogaster*, Le Bourg and Lints (1992) studied, just after flies were removed from the centrifuge and in cross-sectional studies, three behavioral characters known to be affected by aging. Their rationale was

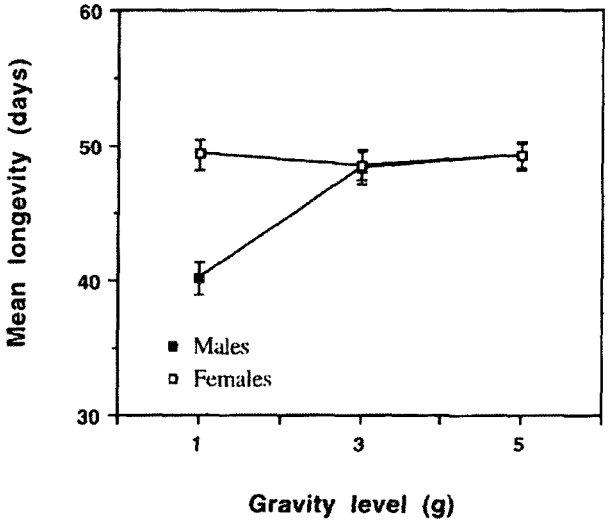

Fig. 3. Mean longevity (± SEM) at 25℃ of *D. melanogaster* flies which have lived at 1 *g* for their whole life or have lived from days 2 to 15 of imaginal life at 3 or 5 *g*, before transfer to 1 *g* for the remainder of their life.

that, if HG-kept flies age faster than 1-*g* flies, age-related changes should be observed at a younger age in HG-kept flies than in 1-*g* flies, with no clear effect of gravity at young age. Climbing activity, i.e., the maximal height reached by a fly in a vial during a 20-s period after cessation of automatic shaking, is impaired in old flies: it decreased at a higher rate with age in 3-*g* and 5-*g* flies than in 1-*g* flies. The paths of young flies released in the center of an arena with no visual target are rather linear and become more sinuous in older flies: the paths of HG-kept flies (2.16, 3.02, 3.97, and 5.02 *g*) became sinuous at a younger age than those of 1-*g* flies. Finally, the spontaneous locomotor activity decreases with age: the activity of 5-*g* flies decreased with age to a higher extent than that of 1-*g* and 3-*g* flies. In summary, the HG-kept flies displayed the same age-related changes with the 1-*g* flies, but these changes were observed at a younger age, where no gravity effect was observed. This whole set of results suggests that aging is sped up in HG. It is of interest to note that effects of HG on behavior can be observed at HG levels at which no longevity decrease is observed.

The previously studied behavioral traits are various features of locomotor activity: spontaneous activity, reactive activity to a mechanical stimulus (climbing activity), or patterns of movement. It may be wondered whether other traits would be also affected by HG. Thus, Le Bourg (1996) studied the proboscis extension response threshold to sucrose of fasted male flies. In accordance with results of age-related changes on threshold (review in Le Bourg, 1996), the threshold increased with age but, unexpectedly, no effect of gravity was observed at any age. As a last step of these studies of behavior of flies kept in HG throughout

lifespan, Minois and Le Bourg (1997) reported that habituation and learning speeds, which decrease with age, were lower in male flies submitted to HG for 1 wk, while no gravity effect was observed in flies kept in HG for 7 wk. After this study was completed, Le Bourg showed that, 5 d after the removal from the centrifuge, young male HG flies still had a lower habituation speed than the 1-*g* flies. These results showed that, as far as learning and habituation are concerned, the HG flies did not age faster than the 1-*g* flies. Rather, it was concluded that HG may act as a mild stress to which flies adapt.

This conclusion prompted the need to study the effect of short HG exposures. Le Bourg and Minois (1997) reported that flies of both sexes submitted to HG for 1 or 4 wk from the second day of imaginal life survived longer at 37°C than flies kept at 1-*g*; however, this positive effect was not observed if flies spent 7 wk in HG before their thermotolerance was recorded. In other words, a short exposure to HG-a mild stress-increased resistance to a more acute stressor-heat. The study of behavioral traits affected by aging showed that flies that had spent 2 wk in HG at young age could age slower than the 1-*g* flies. The HG flies had worse scores on removal from centrifuge than those kept at 1 *g*, but when they aged, they got either similar or better scores than the 1-*g* flies (Le Bourg & Minois, 1999). The study of the mechanisms explaining these effects is still underway, but it seems that heat-shock proteins are at play, in accordance with the induction of heat-shock proteins by heat and other stresses, such as exercise in rats (Skidmore et al., 1995).

Conclusions

Concerning the study of aging in µG, we feel that future experiments should be more rigorous. It is difficult to accept that only limited results with no statistical analysis are reported at a time when research budgets are decreasing from year to year. One problem concerns the use of proper control groups. Performing µG experiments requires the use of several control groups: a 1-*g* group in the spacecraft, 1-*g* and 1.4-*g* groups on ground. The first group allows the comparison of 0 *g* and 1 *g* in conditions where all other factors linked to the flight remain equal. It should be recalled that the 1-*g* group is submitted to HG episodes at launch, and to 0 *g* if the centrifuge is stopped during the flight. The 1-*g* group on ground is the usual maintenance condition; it allows for assessing the effects of the space flight conditions, gravity level being constant. Comparison of 1-*g* groups on ground and in space could allow the detection of uncontrolled differences and must be a preliminary step before analyzing the effect of µG on the traits under study. In the same way, the 1.4 group on ground controls for the possible effects of centrifugation on the results of the 1-*g* space group. If these three control groups do not behave identically, we have to be cautious about what we call an effect of µG itself. Perhaps this "effect of µG" has nothing to do with µG. Furthermore, it could be useful to have another control group submitted to transport between the lab and the cosmodrom.

References

Benguria A, Grande E, and De Juan E (1996) Microgravity effects on *Drosophila melanogaster* behavior and aging: implications of the IML-2 experiment. *J Biotechnol* 47: 191-201.

Bucker H, Facius R, and Horneck G (1986) Embryogenesis and organogenesis of *Carausius morosus* under spaceflight conditions. *Adv Space Res* 6: 115-124.

Economos AC (1982) Human homeostasis in the space environment: a systems synthesis approach. In: Miquel J and Economos AC (eds). Space Gerontology. NASA Conference Publication 2248, Washington D.C., pp 13-35

Ewing AW (1963) Attempts to select for spontaneous activity in *Drosophila melanogaster*. *Anim Behav* 11: 369-378.

Frolkis VV (1993) Stress-age syndrome. *Mech Ageing Dev* 69: 93-107.

Khazaeli AA, Tatar M, Pletcher SD, and Curtsinger JW (1997) Heat-induced longevity extension in *Drosophila*: heat treatment, mortality, and thermotolerance. *J Gerontol Biol Sci* 52A: B48-B52. EMBASE.

Le Bourg E (1996) Hypergravity and aging in *Drosophila melanogaster*. 8. Proboscis-extension-response to sucrose. *Gerontology* 42: 235-240.

Le Bourg E and Lints FA (1992) Hypergravity and aging in *Drosophila melanogaster*. 4. Climbing activity. *Gerontology* 38: 59-64.

Le Bourg E and Minois N (1997) Increased longevity and resistance to heat shock in *Drosophila melanogaster* flies exposed to hypergravity. *C R Acad Sci Paris* 320: 215-221.

Le Bourg E and Minois N (1999) A mild stress, hypergravity exposure, postpones behavioral aging in *Drosophila melanogaster*. *Exp Gerontol* 34: 000-000.

Lee RE, Bryant EH, and Baust JG (1985) Fecundity and longevity of houseflies after space flight. *Experientia* 41: 1191-1192.

Lints FA, Bullens P, and Le Bourg E (1993) Hypergravity and aging in *Drosophila melanogaster*. 7. New longevity data. *Exp Gerontol* 28: 611-615.

Lints FA and Le Bourg E (1989) Hypergravity and ageing in *Drosophila melanogaster*. 7. Fecundity. *Gerontology* 35: 235-243.

Marco R, Vernos I, Gonzalez J, and Calleja M (1986) Embryogenesis and aging of *Drosophila melanogaster* flown in the space shuttle. *Naturwissenschaft* 73: 431-432.

Minois N and Le Bourg E (1997) Hypergravity and aging in *Drosophila melanogaster*. 9. Conditioned suppression and habituation of the proboscis extension response. *Aging Clin Exp Res* 9: 281-291.

Miquel J and Economos AC (1982) Preface. In Miquel J and Economos AC (eds). Space Gerontology, NASA Conference Publication 2248, Washington D.C., p 7.

Miquel J and Philpott DE (1978) Effects of weightlessness on the genetics and aging of *Drosophila melanogaster*. In Rosenzweig SN and Souza KA (eds). Final Reports of U.S. Experiments Flown on the Soviet Satellite Cosmos 936. NASA Technical Memorandum 78526, Washington D.C., pp 32-54.

Miquel J, Philpott DE, Lundgren PR, Binnard R, and Turnbill CE (1978) Effects of weightlessness on the embryonic development and aging of *Drosophila*. In Rosenzweig SN and Souza KA (eds). Final Reports of U.S. Experiments Flown on the Soviet Satellite Cosmos 782. NASA Technical Memorandum 78525, Washington D.C., pp 382-404.

Pearl R (1928) The Rate of Living. University of London Press, London.
Pearl R, Allen L, and Pennimann WBD (1926) Culture media for *Drosophila*. II. A new synthetic medium and its influence on fertility at different densities of population. *Am Nat* 60: 357-366.
Skidmore R, Gutteriez JA, Guerriero V, and Kregel KC (1995) HSP70 induction during exercise and heat stress in rats: role of internal temperature. *Am J Physiol* 268: R92-R97.
Souza KA, Black SD, and Wassersug RJ (1995) Amphibian development in the virtual absence of gravity. *Proc Natl Acad Sci USA* 92: 1975-1978.

[Received June 24, 2000; accepted July 22, 2000]