Hypergravity: Its effect on the estrous cycle of rats

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Summary. Hypergravity affects the estrous cycle of rats. It is prolonged by a force of 1.39 G–1.65 G, and shows irregularities between 1.02 and 1.19 G. Rats centrifuged for 40 days and returned to normal gravity presented a normal estrous pattern.

Various pathological changes have been reported to occur in animals kept under persistent centrifugation, i.e. hypergravity[2]. Ishay and Barr-Nea[3] studied the effects of relatively moderate hypergravity on fertility, delivery and survival of rats. The estrous cycle of rats has been shown to become desynchronized by stress induced via electrical shock[4]. The present study describes the effects of gravitational forces ranging from 1.0 G to 1.65 G on the estrous cycle (EC) of rats. These forces are well within the range of the physiological adaptation of rats[5].

Test design. A special 4-armed centrifuge was used to provide the added gravitational force. The rats were kept in pairs in ordinary breeding cages that were placed at various distances along the arms of the centrifuge, so as to be exposed to different gravitational forces. The cages were tilted at an angle calculated to ensure that the resultant gravitational force was acting perpendicularly on the floor of each cage. In this manner additional stress due to uncomfortable position of the rats was avoided. The resultant forces, with a rotation frequency of 28 rpm, were as described in the table.

Group	No. of rats	Distance from the center (r) in cm	Gravitational force (F) in G
1	4	150	1.65
2	4	132	1.53
3	6	110	1.39
4	2	74	1.19
5	4	20	1.02 (rotating control)
6	6	–	1.00 (stationary control)

The force was calculated according to the formula $F = G \times \sqrt{1 + \frac{(2\pi f)^4}{g^2} \times r^2}$, where G = mg, m = mass, g = gravity constant, f = rotation frequency.

The rats used were 3–4 months old virgin Charles Rivers rats. They were kept at a constant temperature of 25 °C under automatically controlled fluorescent illumination (period of lights: 5.00–19.00 h), and fed on Purina chow and water ad lib. The stage of their estrous cycle was determined by daily vaginal smears[6]. Once daily, but at different times (between 15.00 and 22.00 h), the centrifuge was stopped for 20 min in order to take the vaginal smears, supply food and water, clean the cages and service the machine.

Experiment 1: All rats were kept for approximately 2 EC's (10 days) outside the centrifuge to determine their normal estrous cycle and to accustom them to the procedures. Then 20 of the rats were placed on the centrifuge, as described, for approximately 4 EC's (20 days), and 6 were kept as stationary controls under the centrifuge.

Experiment 2: A study of the after effect of hypergravity: A group of 8 rats was kept rotating for about 8 EC's (40 days) at 1.65 G, i.e. the highest gravity tested. This was done in the same conditions as described in experiment 1, and daily vaginal smears were taken and examined. After spinning, the rats were returned to normal gravity (1.00 G), while still in their original cages, and their estrous cycles were recorded over the next 30 days.

Results. The figure presents the typical pattern of estrous cycles in each group of rats. At 1.65 G (group 1) the spun rats showed a tendency to remain in diestrus for about

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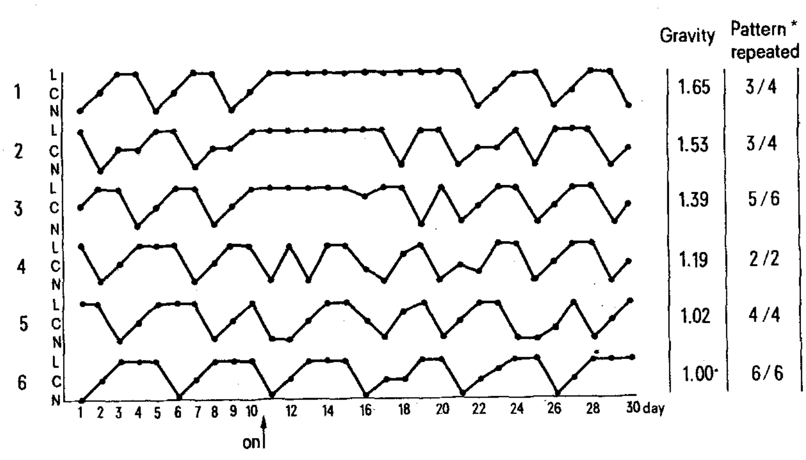

Representative patterns of the estrous cycles in groups of rats before and during exposure to different degrees of hypergravity. Spinning was started on the evening of the 10th day. Groups 1–3 show a prolonged diestrus during the first days of rotation and then revert to normal. Group 4 and the rotating control group 5 show only slight changes in their cycles. Group 6 is the stationary control.
L, diestrus; C, estrus; N, proestrus; * number of rats showing the representative pattern; →, start of spinning for groups 1–5.

10 days, but thereafter reverted to normal cycle while still spinning. This characteristic pattern was repeated in 3 rats out of 4. In group 2 (1.53 G) the tendency was similar but the diestrus was somewhat shorter. Again the pattern was repeated in 3 out of 4 rats. The same pattern was still evident in group 3 (1.39 G) in 5 out of 6 rats, but at a gravitation of 1.19 G or less (groups 4 and 5) the diestrus was not prolonged, and there was only a slight irregularity of the estrous cycles as compared to the stationary control (group 6). In experiment 2 the 8 rats that were spun for 40 days at 1.65 G showed during the last 10 days on the centrifuge normal estrous cycles. Upon return to ordinary gravity, these rats not only persisted in having normal estrous cycles but, from the start, the cycles were highly regular and in 5 out of the 8 rats they were even synchronous. Regularity of the cycles in the experimental group was in fact more precise than in the control group that had never been exposed to centrifugation.

Discussion. These experiments show that rats subjected to persistent moderate hypergravity enter a phase of prolonged diestrus. Within the range of 1.39–1.65 G, the length of the diestrus is positively correlated with the strength of the gravitational force but at a gravity of 1.19 G or less, there is only a slight irregularity of the cycle and no prolongation of the diestrus. We believe that this prolongation effect is purely due to hypergravity, because other kinds of stress, like food and water deprivation, immobilization or change of environment have been shown to produce effects of a different character and duration[7–10]. Moreover, other forms of stress would have had an equal effect on all the rats regardless of their position on the centrifuge, and this was not the case. Our findings are consistent with the changes observed in pregnant rats under similar conditions[3], and also confirm that there is a physiological adaptation with time in the gravity range used[5]. Results of the second experiment are particularly interesting in that they seem to show that a gravitational force of 1.65 G has a stabilizing and synchronizing effect on the estrous cycle of rats. Since this was the only hypergravity tested, it is not yet clear what is the minimal additional gravity and duration needed to induce the stabilizing and synchronizing effects. Further studies are now in progress concerning hormonal changes in rats under conditions of hypergravity[11].

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11 Submitted by E. Megory as a partial fulfilment for the MD degree of Sackler School of Medicine Tel Aviv University.

Testicular blood flow and oxygen tension in unilaterally orchidectomized rats[1]

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Summary. Testicular blood flow was measured by means of Xenon-133 clearance and oxygen tension was measured polarographically in rats 3 weeks after unilateral orchidectomy. There was no difference between experimental and control groups for any of the two parameters.

We have recently shown that endocrine compensation in unilaterally orchidectomized rats (UOR) occurs within 3 weeks of the operation[2]. This compensation is not related to compensatory testicular hypertrophy (CTH), since CTH seems to be a result of the trophic action of FSH, rather than LH, on the seminiferous tubules[3]. Wurtman[4] has shown that LH increases ovarian blood flow and in 1950 Hartman et al.[5] developed a pregnancy test based on the vasodilatation response of rat testes to HCG. Furthermore it has been shown that there is a relationship between the arterial blood flow to the testis and its ability to secrete testosterone under the influence of HCG[6]. We have measured capillary blood flow in the remaining testis of UOR by means of Xenon-133 washout technique in order to establish if the observed endocrine compensation may be due to increased blood flow as a result of elevated LH concentrations[7].

Cross and Silver[8] used polarographically measured oxygen tension to study local and systemic mechanisms that appear to exercise a regulatory influence on testicular circulation. They stated that tissue oxygen tension is largely determined by capillary blood flow. In the present study, we have measured testicular oxygen tension to establish if there are any major metabolic changes in the

Testicular blood flow and oxygen tension in unilaterally orchidectomized and control rats

Method	Unilateral orchidectomy, $\bar{x} \pm$ SEM (n)	Controls, $\bar{x} \pm$ SEM (n)	Significance according to Wilcoxon's two-sample t-test based on range
Xenon-133 clearance (ml/min 100 g)	17.0 ± 1.2 (10)	17.1 ± 1.2 (10)	n.s.
Oxygen tension (pO_2 mm Hg)	21.6 ± 4.0 (7)	17.4 ± 2.1 (8)	n.s.

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