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Liver Regeneration in Rats Exposed to Simulated Changes in Gravity. (31929)

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The biological role of gravity is being investigated in many laboratories as a result of the advent of space exploration. Many studies are aimed at predicting the effects of weightlessness on biological processes, but one must not disregard effects of other changed gravitational conditions such as decreased and increased gravity.

Increased gravity can be simulated in the laboratory by changing the accelerative force with a centrifuge. This technique has been used predominantly for following the responses of animals, including man, to increased gravity. Information on how gravity affects cellular development and growth is sparse. Pflüger(1) and Schultze(2) showed that normal gravity influences the direction of cleavage and development in frog's egg.

The liver is an excellent choice as a mammalian tissue for study of the regenerative process. When two-thirds of the rat liver is removed by surgical ablation, the remaining liver grows until it attains its original mass. The completion of this regenerative process takes 10-15 days(3); however, most of the original mass has been regenerated within 7 days. Information from liver regeneration studies may help in the understanding of general processes of regeneration and growth of other tissues, such as muscle or skin, in the process of wound healing. The present study describes effects of simulated changes in gravity on liver regeneration as measured by mitotic count and on the gross appearance of the regenerating tissue.

Methods. A 10-radial-armed centrifuge having an effective operating radius of 4.5 feet was employed in this study. The cages ($20 \times 10 \times 10$ inches) were mounted in the swing-bucket fashion, allowing for one degree of freedom. The cages swung outward upon centrifugation so that the resultant G-vector was always perpendicular to the cage floor. The centrifuge was illuminated with fluorescent light automatically set for on-off cycling at 6:00 A.M. and 6:00 P.M., respectively. Centrifuged as well as noncentrifuged animals were exposed to these light conditions. The centrifuge was run continuously except for stoppages of no longer than 30 minutes each, 3 times a week, to feed the rats and clean the cages. All animals were kept in air-conditioned rooms.

Three-week- and 7-week-old male Sprague-Dawley rats obtained from Simonsen Laboratory, Gilroy, California, were used. The animals were divided into 3 different groups, 2 of which were noncentrifuged and served as controls to the third group, which was centrifuged. One control group was given food and water *ad libitum* and the other control group was pair-fed with the centrifuged group.

Partial hepatectomies were performed according to the procedure of Ralli and Dumm (4) on both control and experimental animals. The operations were carried out between the hours of 8:30 A.M. and 10:30 A.M. in order to control the effects of diurnal variation in mitosis(5). Surgery was performed under ether anesthesia. Normally, an abdominal incision is made to expose the liver(6); however, since the additional stress of increased gravity on the abdominal wall may interfere with the normal healing of such a wound, we chose to make a dorsal incision(4). On the basis of measurements made after autopsy on a separate group of

10 animals, results showed that 67.2 ± 0.3%* of the total liver was removed by excision of the median and left lateral lobes.

The animals were decapitated and thoroughly bled before the liver was excised. All animals were fasted approximately 16 hours prior to both operation and sacrifice in order to eliminate any differences in food intake during these time periods and to reduce the hepatic glycogen content and thereby facilitate the reading of the tissue preparations. Immediately after sacrifice, a thin slice of liver extending from base to apex was taken from the anterior lobule of the right lateral lobe(7) and fixed in 10% neutral buffered formalin. The tissue was embedded in paraffin, sectioned at 6 μ, and stained with hematoxylin and eosin.

With the aid of a square eyepiece graticule, mitoses in parenchymal cells were counted in histologic sections at a magnification of 450 times. The results were based on counts of 15,000 to 25,000 parenchymal nuclei (approximately 200 fields) for each animal and were expressed as the number of mitoses per 100 parenchymal nuclei, or mitotic index.

To establish the normal rate of mitotic activity in the liver, a group of 8 animals, 7 weeks of age, was sacrificed and mitotic indices were calculated. This age group was selected since all final results in this study were based on 7-week-old animals. We found that the basal mitotic activity of the liver of these animals was approximately 0.01%; this value has been reported previously by other investigators(8).

To determine the effects of altered gravity on mitosis, the study was divided into 3 phases.

Phase I. The animals were operated on in groups of 4 and placed on the centrifuge, 1 group to a cage, within an hour after surgery. They were centrifuged at 2.5 *g* for varying time intervals, removed from the centrifuge and sacrificed 20, 24, 28, 36, 48, 72, and 96 hours after surgery. The time between removal from the centrifuge and sacrifice was always less than 1 hour.

Phase II. The experimental design was the same as in the first phase except (a), the animals were exposed to a higher *g* load (4.7 *g*) and (b), only the 28- and 36-hour postoperative periods were studied, since we felt that any change in mitotic activity as a result of the increased *g* load would show up during these 2 dynamic time periods.

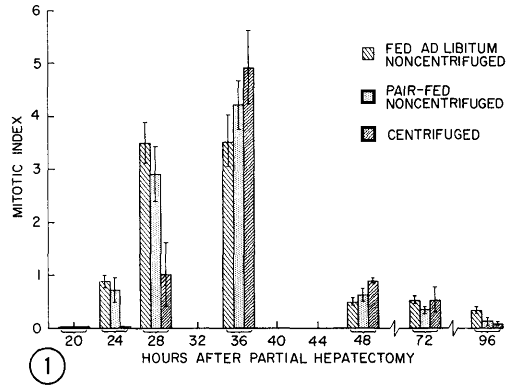
Phase III. In previous studies(9), rats centrifuged at 4.7 *g* appeared to become acclimated to this higher gravitational environment in less than 2 weeks since at that time their food and water consumption and growth curves returned to normal. For the third phase, a 4-week period of centrifugation to condition the rats to 4.7 *g* was selected so that the 3-week-old weanling rats were 7 weeks old when they were removed from the centrifuge and subsequently subjected to surgery. In this manner, the rats in this and the previous experiments were the same age at the time of operation. The 4.7 *g*-conditioned rats were hepatectomized within an hour after removal from the centrifuge and kept at normal gravity until they were sacrificed 24, 28, and 36 hours after the operations. Despite the double stress of surgery and centrifugation, no deaths occurred.

Results. Phase I. The mitotic indices of the 2 noncentrifuged groups and the 2.5 *g*-centrifuged group are shown in Fig. 1. At 20 hours† after partial hepatectomy, no increase in the mitotic index above the normal value of resting liver tissue (0.01%) was noted in any of the 3 groups. At 24 hours, the centrifuged group, which was still mitotically inactive, displayed a mitotic index that was significantly lower ($P<0.001$) than that of either of the 2 noncentrifuged groups as judged by the *t* test. The mitotic index of the pair-fed control group at this time period was 0.72 ± 0.24‡ compared to that of the centrifuged group of 0.005 ± 0.003. At 28 hours, the centrifuged group became mitotically active but still displayed a lower mitotic index which was 64% less than that of the control group ($P<0.05$). During the following time intervals, no significant difference

*Mean ± standard error.

†The animals were centrifuged approximately 1.5 hours less than the indicated postoperative time period because of the time lag between operation and centrifugation and centrifugation and sacrifice.

‡Mean ± standard error.



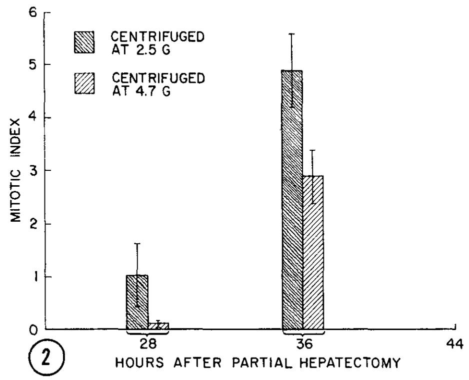


FIG. 1. Liver regeneration in rats exposed to 2.5 *g*. The mitotic index is the number of mitoses per 100 parenchymal nuclei. Each value represents the mean ± S.E. of 12 to 16 animals.

FIG. 2. Comparison of regeneration in rats exposed to 2.5 *g* and 4.7 *g*. Each value represents the mean ± S.E. of 12 to 16 animals.

was noted in the mitotic indices of the centrifuged and noncentrifuged groups. The data indicate that the onset of mitosis is delayed in the centrifuged group.

There was no significant difference in the mitotic indices of the pair-fed and *ad libitum* noncentrifuged groups even though the former group took in 45% less food than the latter group during the 4-day period. Because of the lack of difference in the mitotic indices of the 2 noncentrifuged groups, the pair-fed noncentrifuged group was chosen as the only control group in the following experiments. This group more closely approached the conditions of the centrifuged group.

Phase II. Liver regeneration in rats centrifuged at 4.7 *g* was compared to that of the rats that had been centrifuged at 2.5 *g* in the previous phase. The mitotic indices of these 2 groups are shown in Fig. 2. A pair-fed noncentrifuged group not shown on the graph served as a control for the 4.7 *g*-centrifuged group and compared favorably with the pair-fed control group (shown in Fig. 1) for the 2.5 *g*-centrifuged group. The 4.7 *g*-centrifuged group showed a lower mitotic index during both the 28- and 36-hour time periods. The difference was significant ($P<0.05$) at the 36-hour time interval and represented a decrease of 42% in the mitotic index. These results suggest that increasing the *g* load from 2.5 *g* to 4.7 *g* will further delay the onset of mitosis.

Phase III. Liver regeneration in rats previously conditioned to a 4.7 *g* environment for 4 weeks was compared to that of a group of pair-fed noncentrifuged animals. The 4.7 *g*-conditioned group displayed a higher mitotic index than the pair-fed controls during the 3 time periods studied (Fig. 3). The difference was significant ($P<0.01$) at the 28- and 36-hour time intervals in which increases in the mitotic index of 140% and 70%, respectively, were observed.

The higher mitotic activity observed in the 4.7 *g*-conditioned rats indicates that conditioning enhances mitosis and, although not experimentally proven, the data suggest that an earlier onset of mitosis occurs.

The mitotic response of the pair-fed noncentrifuged controls in Fig. 3 was similar in pattern to that of the pair-fed noncentrifuged group in Fig. 1 but proportionally smaller. This difference in magnitude may have been caused by the difference in time periods of pair feeding. The control group in Fig. 1

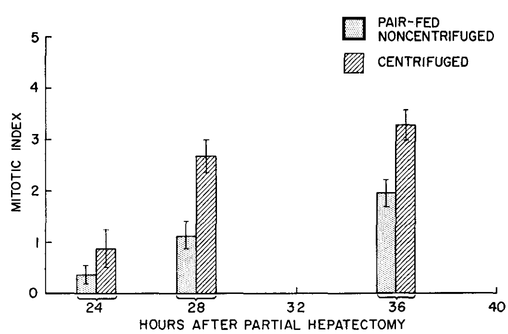


FIG. 3. Liver regeneration at normal gravity in rats previously conditioned to 4.7 *g* for 4 weeks. Each value represents the mean ± S.E. of 9 to 12 animals.

was pair fed for periods up to 4 days, while the control group in Fig. 3 was pair fed for more than 4 weeks.

Discussion. The objective of this study was to determine whether the stress produced by exposing rats to simulated changes in gravity altered the regenerative process in liver following partial hepatectomy. Other work on the effects of stress on liver regeneration includes that of Beckman and coworkers(10) who found that exposing monkeys to 40 *g* caused a foci of degeneration and regeneration in the liver. Kotovskiy(11) described general changes in the morphology of the liver obtained from dogs exposed to 8 *g*. Other investigators have demonstrated that stresses such as fatigue from prolonged swimming (12), irradiation(3), or 82% subtotal hepatectomy(13) reduced the production of DNA in the regenerating rat liver. In the latter 2 studies, delay in the initiation of mitosis has also been shown to occur.

The results of the present study indicate that centrifugation stress delays the initiation of mitosis and that the delay is proportional to the intensity of the stress. The results also suggest that mitosis was enhanced in the group of animals that was conditioned to an increased gravity; however, this last study must be interpreted with caution. In approximately half the rats that were exposed to 4.7 *g* for 4 weeks, the caudate lobe of the liver was necrotic. The uniqueness of the observation was that only the caudate lobe was involved. Therefore, its position in relation to the rest of the liver and other tissue must have been a factor in causing this condition.

Preliminary results of a study to resolve this problem suggest that the necrosis observed in the caudate lobe is probably due to severe ischemia. If a force such as a high *g* load were applied dorsoventrally to the animal, the caudate lobe could be compressed by surrounding tissue so that the circulation at the pedicle of the lobe would be obstructed, producing ischemic necrosis.

Several facts have led to the question, does centrifugation *per se* enhance mitosis in the 4.7 *g*-conditioned rats or is the degenerative condition in the liver, which is brought on by centrifugation, the inducer? (a) The mitotic indices of the normal appearing liver and the necrotic liver of the 4.7 *g*-conditioned rats were similar after two-thirds partial hepatectomy, and (b) when 15 of the normal appearing unoperated livers of the 4.7 *g*-conditioned rats were examined microscopically, 6 of them showed a higher than normal mitotic rate (0.15%). Experiments to resolve there problems are now in progress.

Summary. Partial hepatectomies were performed on 7-week-old male Sprague-Dawley rats that were subsequently centrifuged at 2.5 *g* or 4.7 *g* and sacrificed at various postoperative time intervals. No liver regeneration as measured by the mitotic index was noted in rats centrifuged at 2.5 *g*, 24 hours after operation; at this time period the control rats at normal gravity showed initiation of mitotic activity. At the 28-hour period, regeneration at 2.5 *g* was noted but the value was significantly lower than that of controls. The initiation of mitosis was delayed even longer in the group of animals that was centrifuged at 4.7 *g*.

To study the effects on liver regeneration of a decrease in gravity, 3-week-old weanling rats were centrifuged for 4 weeks at 4.7 *g*, removed from the centrifuge, hepatectomized, and sacrificed 24, 28, and 36 hours after partial hepatectomy. Greater mitotic activity was observed in the 4.7 *g*-conditioned rats than in the rats not exposed to centrifugation during all 3 postoperative time periods. Thus, the onset of mitosis is delayed in rats exposed to increased gravity and mitosis is enhanced in rats conditioned to increased gravity when they are subsequently returned to normal gravity.

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Ecology of Plague in Vietnam I. Role of *Suncus murinus*. (31930)

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The importance of the insectivore *Suncus murinus* as a reservoir for plague has been a matter of concern for a number of years. In 1914, Kerandel(1) isolated *Pasteurella pestis* from *S. murinus* in Phnom-Penh, Cambodia. He noted that specimens trapped in this area harbored numerous rat fleas, *Xenopsylla cheopis,* and concluded that since the shrew shared fleas with the more common plague carriers that it might serve as an unrecognized reservoir.

In 1932, Kopstein(2-5) reported the results of a survey of 10,000 houses in Java. He found *S. murinus* to be a village dweller living in close association with *Rattus rattus,* both within and outside the dwellings. Approximately half of the *S. murinus* were found to be carriers of *X. cheopis*. He believed that while *S. murinus* may play a role in transmission of plague among rodents, its lack of contact with man excluded it as an important factor in human plague. Since it was found exclusively in the villages, he doubted the shrew played any part in the transmission of plague from village to village. Schuurman (6,7) disagreed with Kopstein concerning the role of *S. murinus* in the epidemiology of plague. He believed that the shrews were active migrators and as such could play a role in intervillage transmission.

Suncus murinus trapped during the 1922-1930 plague outbreak in Macassar were found infested with both *Xenopsylla astia* and *S. cheopis,* the first species predominating (Van Der Walle(8)).

Sharif and Narasimhan(9), as quoted by Pollitzer(10), stressed the importance of *S. murinus* wandering from house to house as well as undertaking excursions from village to village in India. Pollitzer further stressed their role in conveying the rat ectoparasite into human habitations.

Suncus murinus has been recognized as a potential reservoir in China by Pollitzer(11) in 1948 and by Yang *et al*(12) in 1939. In a review of plague in China by Kraminskiy(13), he reported that the shrew was often found in the plague infected villages of Fukien. *Pasteurella pestis* was isolated from 1 of 262 *S. murinus* trapped in the Yunnan foci.

Herivaux and Toumanoff(14) noted an increase of *S. murinus* in the Saigon outbreak of 1941-43. However, records of the Institute Pasteur, Saigon reveal no isolations of *P. pestis* from this species in the various surveys conducted in Vietnam.

Materials and methods. As part of the plague investigation in Vietnam, a program of animal trapping was carried out. Live box traps were set out each night in the Saigon-Cholon area and at varying intervals in other geographic areas of Vietnam. The traps were

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